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CONTENTS

THE SKULL AND THE TEMPORAL REGION OF THE LIMBLESS LIZARD *OPHISAURUS GRACILIS* (GRAY)*

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GERVAIS (1853) pointed out acrodont dentition in *Trogonophus* and allied genera of Amphisbaenidae. Zimmermann's (1913) description on the chondrocranium of *Anguis fragilis* is on the whole similar to the ones on that of *Lacerta* by Parker (1880) and *Eumeces* by Rice (1921). The down growths of the frontals and parietals which form the anterior brain case subsequently lead to the disappearance of the inter-orbital septum. This is not particular to snakes only but has been found in burrowing lizards such as *Acontias meleagris* (Brock, 1941) and even in some *Gekkos* and *Varanids* by Bellairs and Underwood (1951). The orbito-temporal region of snakes is greatly modified and resembles that of the burrowing lizards *viz. Anguis* (Brock, 1941). Zangerl (1944) recorded appreciable variations in his outstanding contribution on the osteology of the skull of *Amphisbaenia* and *Geocalamus*. He observed separate azygous parietal from the paired frontals whereas in *Bipes biporous* not only paired parietals exist but they are united with the frontals, resulting in the formation of a large palate. In *Bipes* the pterygoid also unites with the palatine and maxilla. In *Amphisbaenia*, however, the limitation of the palatine is clearly marked.

In the limbless lizard *Monopeltis capensis* (Kritzinger, 1946) parietals do not extend as far back as in snakes but the anterior brain case is very much the same. Bellairs (1950) and Toerien (1950) accounted for the cranial morphology of *Anniella* and briefly compared it with that of other burrowing lizards. It is however noteworthy that the skull of limbless lizards and snakes resemble each other to a great extent irrespective of their mode of origin.

A study, therefore, of the solitary genus of an Indian variety of Anguidae, *Ophisaurus gracilis* along these lines should be useful.

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* Contribution No. 13.

Material and Technique

The specimens of *Ophisaurus gracilis* were procured from Darjeeling. A few alizarin preparations of these lizards were used for studying the general features of the skull. For closer examination, a few skulls were cleaned by maceration and a few others by boiling in a 5% solution of ammonium carbonate (Cantuel, 1949). The alizarin stained ones were cleared by Hollister's (1934) method.

Observations

The skull of *O. gracilis* or the " Burmese Glass Snake " is so modified by undergoing reduction that it approaches the ophidian type; but there are a number of other cranial characters which give it a normal saurian form too. Besides this the skull is streptostylic; modified parapsidan or degenerated synapsidan; mesokinetic and tropotrabic. It measures 0.7 cms. approximately from the azygous prexamilla to the occipital condyle. Its width between the temporal space of either sides is 0.3 cms. while its height from the lower jaw to the parietals above is 0.2 cms. The skull is pyramidal in shape and is characterized by the presence of a jugal, the postorbital and the postfronto-squamosal arches, which are generally absent in the allied families of the limbless lizard. The occipital region is fused into a bony ring like structure. The supraoccipital and the pro-otic undergo excessive development with which the post-temporal fossa disappears. The supratemporal fossa is being replaced by the dermal bones as the parietals and the postfrontal channel. The only representation of the temporal space is by the infratemporal fossa. The constituents of the bony palate have fibrous connection enabling its movable atriculation. The palatine is fully denticulated as in the Chinese *Ophisaurus (Dopasia) harti* noted by Mcdowell and Bogert (1954). The pterygiod is only partially denticulated. No vomerine teeth exist. The maxilla unites ventrally through the ectopterygoid with the pterygoid, the latter, however, has no connection with the basisphenoid as the basipterygoid is lacking. The members of the circumorbital series are typically lacertilian. The bridge formed by the orbitotemporal processes contributed by those of the postorbital and the jugal, prevents orbit continuation with the adjacent temporal space. The downgrowth of the frontals and parietals eliminates the interorbital septum without causing any change in its *tropobasic* character. Absence of the epipterygoid isolates the movable quardrate. The components of the mandibular ramus though reduced, are similar to

those of other saurians. The splenial is absent, whereas the coronoid persists, the latter resembles a coronoid nodule of Llysildae.

The Cranium :

The cranium is tubular enclosing the brain and continuing posteriorly through the foramen magnum as the vertebral column. The cranium can be studied conveniently under the following heads :

(a) the occipital region, (b) the parietal region and (c) the frontal region. As in snakes (Boulenger, 1890 ; Gadow, 1901 and Haas, 1931) the cranial bones are solidly united or rather the cranium can be said to be " Starres Ganzes " (rigid whole) " einheitlichen Ganzes " (a whole forming one unity).

The occipital region :

The occipital region of the cranium has four constituents the exoccipitals, the basioccipital and the supraoccipital. All these are fused together round the foramen magnum to form a ringlike bony structure and whence the name occipital ring. The fusion of all the four occipital constituents devoid of any suture or fibrous arrangement or even without lateral lines of demarcation is an interesting feature. However, the indentification of the various components is possible with regard to the disparity in their position such as the dorsally lodged supra-occipital (Fig. 1), laterally placed exoccipitals and ventrally disposed basioccipital (Fig. 2). It should be noticed that this fusion among the occipital bones as a solitary bony frame work is characteristic of skinks which may be considered as the degraded forms of unknown phylogeny.

Jan and Sordelli (1870-1881) mentioned in *Typhlops braminus*, the presence of a single fused bone behind the two parietals and regarded it as the fused supraoccipital ; exoccipital and pro otic. Haas (1931) has also shown this fusion in snakes represented by two bones lying posterior to the parietal, which are separated in their turn by a mesial longitudinal suture with one another. Mukerjee and Das (1932) noted these two bones but did not label them. Mahendra (1936) recorded the complete absence of the supraoccipital in *Typhlops braminus*. But in *Ophisaurus* unlike snakes all these bones (supraoccipital, exoccipitals and the pro-otics) have separate identity although they form a solitary ring. The supraoccipital and the pro-otic are very prominent. It is the lateral elevations of these bones which undergo enormous development that the post temporal fossa disappears. The exoccipitals are very minute bones on the lateral

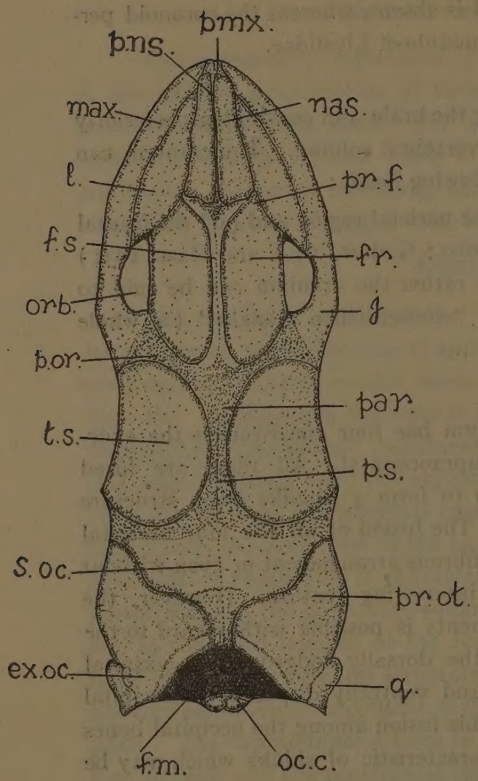

FIG. 1.

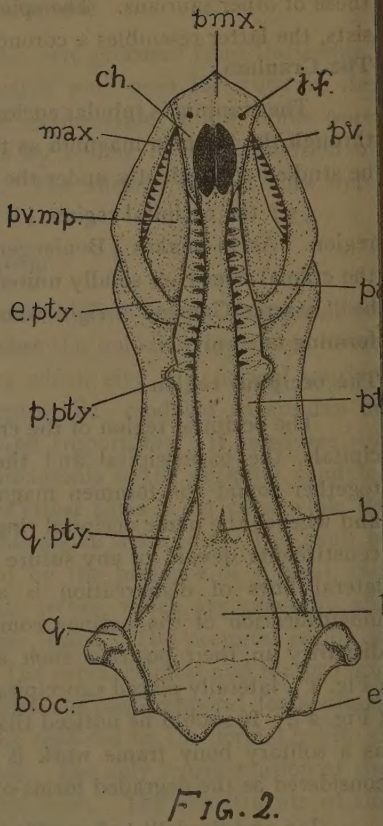

FIG. 2.

Fig. 1.—Dorsal view of the skull of *Ophisaurus gracilis* (Gray)

Fig. 2.—Ventral view of the skull of *Ophisaurus gracilis*

bas. basishenoid; b.oc. basioccipital; b.r. basisphenoidal rostrum; ch. choanae; e.pty. ectop goid; ex. oc. exoccipital; f.m. foreman magnum; f.r. frontal; f.s. frontal suture; j. j jf, Jacobson's organ; l. lacrymal; max. maxilla; nas. nasal; oc. c. occipital condyle; orb. o pal. palatine; par. parietal; p.mx. premaxilla; p.ns. processus nasalis of premaxilla; p.pty. pa pterygoid; p.or. post-orbital; p.rf. prefrontal; pr. ot. pro-otic; p.s. parietal suture; pv. vomer; pv.mp. maxillo-prevomerine process; pty. pterygoid; q. quadrate; q. pty. quad pterygoiod; s.oc. supraoccipital; t.s. temporal space.

sides of the foramen magnum. The exoccipital cannot be differentiated into its paraoccipital part, as in other lizards. The basioccipital is the quadrangular bone bearing no tuberosities to make the tuberculum sphenoccipitale. It, however, approaches anteriorly the hinder extremity of the

basisphenoid with a slight sutural distinction which separates them. The reniform occipital condyle is wholly formed by the basioccipital as described by Kingsley (1925). The triple origin of the occipital condyle postulated by Goodrich (1930) does not hold true in this species.

The parietal region :

Adjacent to the supraoccipital and directed towards the snout of the cranium is the parietal region of which the parietal dorsally and the basisphenoid ventrally are the two important bones. There is a sutural connection present between the parietal and the supraoccipital as in *Amphisbaenidae* and *Chamaeleonidae.*

The parietals (Fig. 1) are paired, separate, unfused structures with a median line of separation just as in *Typhlops* (Jan and Sordelli, 1870-1881 ; Haas, 1931, Mukerjee and Das, 1932 and Mahendra, 1936).

In *Amphisbaenids* such as *Amphisbaena* and *Geocalamus*, Zangerl (1944) recorded a single parietal, which was clearly separated from the paired frontals.

In *Bipes*, however, the parietals and frontals are united together. The constitution of the parietal bone in the skull of snakes and lizards has been a subject of discussion in the past. Gadow (1901) mentioned that in snakes the parietals are always fused into a large unpaired bone resulting in the formation of a sharp crest which overlaps the occipitals. Sedgwick (1905) stated that in serpentes the parietal is unpaired and sends lateral process downwards to meet the basisphenoid. Williston (1925) also held the view " the parietals being always fused in snakes. " Mukerjee and Das (1932) referred to the occurrence of double parietals in *Typhlops* and so also Mahendra (1936).

Paired parietals among lizards are met with in *Hemidactylus* and a few other Geckos ; *Uropletidae* and *Xantusidae* and also in *Sphenodon* (Günther, 1867). The absence of the interparietal foramen or the pineal foramen in *Ophisaurus* is an important difference from other saurians. The down growth of the parietals as well as the frontals as in snakes and a few burrowing lizards eliminates the interorbital septum.

Thus *Ophisaurus* has a paired separate unfused parietal (Fig. 1) as in *Typhlops* (snake) and *Hemidactylus* (lizard) Mahendra (1936 and 1948).

The basisphenoid :

The basisphenoid or the sphenoidum basilare (Fig. 2) is one of the important bones of the ventrally placed occipitosphenoidal part of the skull. It is rectangular in shape and provides a spatulate basisphenoidal rostrum (Fig. 2) which represents the parasphenoid process of *Icthyopsida*. There is no parasphenoid in *Ophisaurus* as also in *Scincus*. Laterally the basisphenoid does not give any basipterygoid process for its articulation with the pterygoid. The absence of the basipterygoid process or the basitrabeculae restricts the movements of the pterygoid with the quadrate bone. The immovability of the basisphenoid with the pterygoid shifts the line of skull movements anteriorly. Thus in *Ophisaurus*, as in snakes, neither the basitrabecular process nor the interorbital septum are present.

The frontal region :

In *Amphisbaenidae* the frontals are generally paired ossifications or they may be fused with the parietals to form one large plate as in *Bipes biporous* Zangerl, (1944). In *ophisaurus* the frontals are paired structures which together with the pre and postfrontals (Fig. 3) comprise the frontal ring of the cranium, lying closely applied to the anterior margin of the parietals. The postfrontals are fused with the postorbitals. This fusion is being represented by a small process of the postorbital directed towards the frontal. Zangerl (1944) observed the complete absence of the post-frontal as well as the postorbital in Amphisbaenian skull. In *Typhlops*, Mahendra (1936) cited fusion of the postfrontal with the postorbital in the same way as it occurs in *Ophisaurus*.

The frontals descend downwards and consequently the inter-orbital septum atrophies and as such the two orbits are being separated by the frontal elevations. The prefrontal together with the fused postfrontal forms the supraorbital arch. From the fused postfrontal there extends towards the posteriorly placed squamosal, an arcade running parallel to the parietal. This is the postfronto-squamosal arcade. In fact it is this arch which in union with the latero-ventral extension of the parietal ob-literates the upper temporal fossa. The two prefrontals are quite apart from one another by the intervention of the paired nasals. Their close approach is of general occurrence in the Scincoid lizards.

The Olfactory Capsule :

The olfactory capsule comprises three pairs of the dermal investing bones : the nasals, the septomaxillaries and the prevomers. Unlike snake

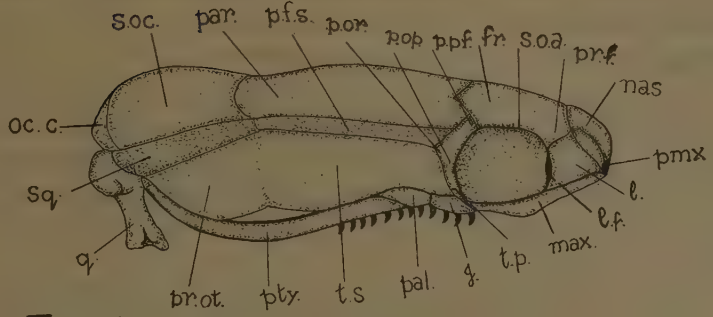

FIG. 3.

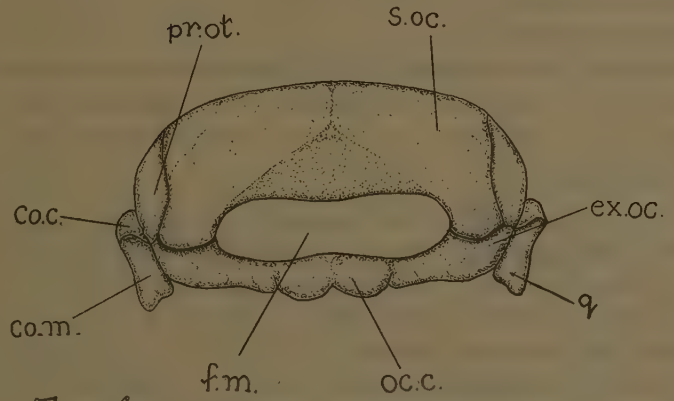

FIG. 4.

Fig. 3—Lateral view of the skull of *Ophisaurus gracilis*
Fig. 4—Posterior view of the skull of *Ophisaurus gracilis*
co. c. condylus cephalicus; co.m. condylus mandibularis; ex. oc exoccipital; f.m. foramen magnum; fr. frontal; j. jugal; l. lacrymal; l.f. foramen lacrymale; max. maxilla; nas. nasal; oc. c. occipital condyle; pal. palatine; par. parietal; p.f.s post-fronto-squamosal; pmx. premaxilla; p op. orbital process; p. or. post-orbital; p. pf. postfrontal process; prt. prefrontal; pr. ot. pro-otic; pty. pterygoid; q. quadrate; s.oa. supraorbital arch; s.oc; supraoccipital; sq. squamosal; tp. temporal process of the jugal; t.s. temporal space.

the nasal capsule is not devoid of any floor. The prevomers bound its ventral limit. The septomaxilla passes through the capsule while the nasal extends along the dorsal surface, but both extend from the premaxilla and abut against the ventral part of the frontal. The nasal and septomaxilla are among the most essential elements which help to main-

tain the rigidity of the nasal capsule in the adult and enable the kinetic movement to take place in the region of the snout.

The nasals :

Paired nasals (Fig. 1) present in *Ophisaurus* are commonly met with in a number of other lizards and snakes. They form the roof of the snout extending from the anterior margin of the premaxilla to the anterior end of the frontals. The processus nasalis or the nasal process of the premaxilla serves to separate the paired nasals, on either side of which are the anterior nares.

The nasal septum is deep and unperforated. This septum helps to maintain the rigidity of the whole nasal capsule. There is no prenasal process as found in a few lizards and snakes.

The septomaxillaries :

The septomaxillaries are only visible on removal of the nasals. They occupy the position shown by Lapage (1928) in reptiles ; *i.e.* forming the floor of the nasal capsule and the roof of the Jacobson's organ.

The prevomers :

The prevomer (Fig. 2) is unpaired in *Lacerta* (de Beer, 1937) but Parker (1880) described them as paired in the same lizard. It is unpaired in *Chalcides* (Haas, 1936) ; *Acontias* (De Villiers, 1939) and *Calotes* (Iyer, 1943). Pearson (1921) described unpaired prevomer in *Lygosoma* embryo. In *Ophisaurus*, however, the prevomers are paired, oblong, plate-like structures, serving as the resting base for the septomaxillaries. The prevomers are quite separate from the pterygoid by an intervention of the palatine. There is no trace of the pterygo-prevomerine contact. The prevomers have no vomerine teeth. Between the prevomer and the premaxilla lie small formina on either side probably as representative of the Jacobson's foramen, followed posteriorly by the slit-like openings, the choanae (the posterior nares).

The Auditory Capsule :

Generally with the closing down of the ear opening the tympanum disappears in lizards. This rule holds true for the limbless lizards also. But as far as this species is concerned, although the ear opening which is sub-circular, is present yet there is a total absence of the tympanum. The disappearance of the tympanum consequently brings no change in the disposition of the slender columella auris, which in spite of extending from

the tympanic membrane to the fenestra ovalis is closely applied to the inner hinder aspect of the quadrate, a condition similar to those of snakes and found in a few other lizards such as *Holobrokia*, *Chamaeleons*, *Scincus*, *Agamids* and also in *Sphenodon* and *Crocodilia*. The columella auris exhibits absence of the process called the stolohyal by Parker (1880); *extra columella* by Gadow (1901); *process internus* by Bäckstörm (1931), extrastapedial structure by Smith (1935). This process usually extends here from the end of the columella to the medial margin of the quadrate. Thus, it is evident that lizards which are on their way of evolution undergo retrogressive evolution too as far as the constituents of the auditory capsule are concerned. Smith (1935) traced the evolutionary changes of the auditory capsule among various groups. He discovered that the skin over the tympanum disappears first, next the tympanum and then extra stapedial structures and finally the columella auris fused with the quadrate. The bones included under this head are the pro-otics; the opisthotics and the supraoccipital.

The pro-otic :

The small size of the brain is responsible for placing the pro-otics (Fig. 3) laterally in the skull. The pro-otic does not amalgamate with supraoccipital and exoccipital, but the reduction and compactness of the cranial constituents in *Ophisaurus* renders the triradiate character of this bone to a simple solitary mass. No distinction, therefore, between the various processes of the pro-otic is possible, on the other hand it undergoes enormous development. This excessive development of the pro-otic along with the equally massive development of supraoccipital result in the closing down of the foramen retro-temporalis of *Siebenrock*. There are, however, no foramina ovalis and rotunda as in snakes, *Sphenodon* and *Testudinata*. The opisthotic is presumed to have been incorporated with the pro-otic and the exoccipital. The supraoccipital is a very prominent bone and does not show any disparity in its position in comparison with the bones (parietals) lying in front of it. The columella cranii descending from the parietal to the pterygoid is absent as in *Amphisbaenidae* (Zangerl, 1944) and also in *Chamaeleonidae* and *Dibanidae*, and hence *Ophisaurus* is a non-kinocraniate lizard as classified by Sedgwick (1905). The absence of the epipterygoid adds to the movability of the streptostylic quadrate by enabling it to be completely separate. The presence of the epipterygoid has been recorded in *Anelytropidae* among the group of limb-

less lizards. According to a few authors this family (*Anelytropidae*) is an artificial assembly of some degraded *Scincoids*.

The Orbito-temporal Region :

The orbito-temporal region of a limbless lizard resembles that of snakes. There are a number of other interesting cranial features of this region which establish their close affinity with lizards particularly those of scincidens.

The skull is typically saurian. The supratemporal fossa is roofed over the post-fronto-squamosal arch which completely replaces it and consequently there is no supratemporal fossa. The supratemporal arcade is contributed both by the postfrontal and the squamosal (Fig. 3). The latter is reported to be absent in amphisbaenids (Zangerl, 1944). The tendency towards the reduction in size of the supratemporal fossa and its complete disappearance have been recorded in scincoid lizards, which too lead a burrowing life, and show reduction of their limbs. The supratemporal or pterotic or tabular is absent as in amphisbaenidae (Zangerl, 1944) and hence it is the squamosal (Fig. 3) and not the supratemporal which ranks next to the parietal in the quadrate articulation of the skull, a condition similar to that of snakes. The massive development of the pro-otic and the supraoccipital of the auditory capsule result in the complete obliteration of the post-temporal fossa and its arcade. The reduction of the foramen retrotemporalis of Siebenrock (1895), due to the enlarged pro-otic has also been observed in skinks. The infratemporal fossa, the sole representative of the temporal space, is very distinct extending from the jugal to the quadrate (Fig. 3). It is bridged over anteriorly by an arch formed by the union of the orbital process of the postorbital and the temporal process of the jugal. The parietal as well as the postfronto squamosal arch bound it above. Posteriorly the isolated (due to the absence of the columella cranii) and streptostylic quadrate limits its boundary while the absence of the quadrato-jugal or the infratemporal arcade enables it to open freely below, though the partially edentulous pterygoid bounds it ventrally. Thus the species in question resembles a *synapsidan* skull in having a single temporal fossa and arcade, but again differs from it and the allied families of the limbless lizards ; *Anelyptropidae, Dibamidae, Anneiellidae, Amphisbaenidae,* and *Pygopodidae* in having the presence of a post latero-frontal (postorbital), the jugal, the orbital and the temporal processes and the post-fronto-squamosal arch which are the possessions of

the *parapsidan* skull. In lizards, in fact there exists no concordance between temporal fossae and the arches which bound them and their inclusion as the modern representative of the modified parapsidan type is only a superficial assemblage for it is difficult to assign them any of the six types of the reptilian skulls recognised by Versluys (1936).

The downgrowths of the frontals and the parietals (not forming a single large plate as in *Bipes*) from the anterior brain case, lead to the disappearance of the inter-orbital septum as in snakes and in few burrowing lizards such as *Acontias* (Brock, 1941) and *Monopeltis* (Kritzinger, 1946) and even in some *Gekkos* and *Varanids* (Bellairs and Underwood, 1951). It is interesting to note that in spite of the absence of the inter-orbital septum the trabeculae cranii fuse immediately in front of the hypophysis and thereby bringing no change in the *tropotrabic* character of the skull. The constituents of the circumorbital series undergo no reduction or atrophy due to the compactness of the bones in this region, on the other hand they are clearly identified by way of the lateral lines to use as reference marks. The orbit is closed in front by a definite lacrymal bone (Fig. 3) separating it from the olfactory capsule. There is a distinct foramen lacrymale at the anterior margin of the optic capsule. Zangerl (1944) observed the complete absence of the lacrymal in *Amphisbaenia, Geocalamus, Trogonophus* and allied genera. The supraorbital arch is formed by the union of the pre- and post-frontals and covers the orbit above. The maxilla and the jugal bound it below, while the dentigerous palatine surrounds it ventrally. Zangerl (1944) reported that the exact contour of the palatine is untraceable in Amphisbaenids for they appear to have united with the pterygoid and maxilla anteriorly as in *Bipes*. Both the maxilla and the palatines in *Ophisaurus* have lateral conical pleurodont teeth in the former they are directed inwards and in the latter outwards in contrast to a simple acrodont dentition of Amphisbaenids accounted by Gervias (1853). The palatal teeth in full are restricted to the palatines alone as in *Oligodon, Dasypeltis* and *Antractaspis*. Posteriorly the orbit bears a distinct mucronated postorbital which sends a process towards the frontal to represent the fusion of the postfrontal with it. DE-villiers (1939) observed in *A. meleagris* only the post-orbital, the postfrontal being absent. In Amphisbaenid skull both the postfrontal and the postorbital are absent and, therefore, the temporal arch is not seen. The post-latero-frontal on the other hand terminates into sharply pointed orbital process which in its turn meets the temporal process of the

jugal (Fig. 3) and, thereby, limits the boundary of the orbit behind averting the confluence of the orbit and the temporal space. The closing of the orbit at the hinder extremity is a common occurrence among lizards. However, in snakes and a few other lizards such as *Hemidactylus* and *Varanus* due to the absence of the proper orbito-temporal processes the orbit coalesce with the temporal space, making it evident that there is a reduction in the temporal space (like snakes) and complete retention of the members of the circumorbital series (like lizards). These pecularities exhibit transitory phase of phylogeny.

The Bony Palate :

The pterygo-prevomerine contact in Rhyncocephalia foreshadows its persistence in skinks and other degraded lizards. Modern lizards reveal only its superficial continuity. In *Ophisaurus*, however, the so called vomers or the prevomers are quite separate. The palatine stands in the way of the pterygoid approach with the prevomer. The following are the paired palatal bones (a) the pterygoid, (b) the palatine, (c) the prevomer and (d) the ectopterygoid (the transpalatine or the transversum).

The ptergygoids :

Zangerl (1944) noticed that in Amphisbaenids though the pterygoid is disposed in the same manner as in other lizards but is completely toothless except in *Bipes* where the pterygoid appears to have united with the palatine and pterygoid. In *Ophisaurus* the pterygoids (Fig. 2) are quite separate and much extended bones, tapering posteriorly on their way towards the inner ventral aspect of the quadrate near the condylus mandibularis. The pterygoid does not bear more than one series of teeth and that too exclusively on one side of it, unlike other species of *Ophisaurus* having 1-3 series of pterygoidean teeth directed at more than one side of the pterygoid. Moreover, the pterygoid in this species is partially denticulated with the lateral conical teeth exhibiting pleurodont dentition, and directed outwardly in contrast to those inwardly directed maxillary teeth. This tooth bearing part of the pterygoid may well be said the palatal part, while the hinder edentulous portion is the quadrate part of the pterygoid. This distinction is followed in obligation of the absence of fossa columella of the epipterygoid and the basipterygoid process. The two pterygoids are apart from one another enclosing a pterygoid vacuity in between them which in its turn continues anteriorly as the palatine vacuity.

The palatines :

The palatines are noticed in all saurian skulls. However, in *Amphisbaenia* the limitation of the palatine is clearly marked. In *Ophisaurus* the palatines are fully toothed. The presence of an entirely denticulated palatine in the palatal region has been observed in a number of ophidians *(oligodon, Dasypeltis* and *Antractaspis).* Each palatine extends from the anterior and of the palatal pterygoid to the ventro-posterior aspect of the azygous premaxilla.

The prevomers are lying at a slightly lower level than other palatal elements. Anteriorly the palatine extends between the prevomer and maxilla to form a short maxillo-palatine apparatus. The palatines for their major part are separated from the maxilla by the posterior palatine or the suborbital vacuity.

The prevomers have already been discussed along with the olfactory capsule.

The ectoperygoids :

The transpalatine or the ectopterygoid joins the maxilla on one side and the pterygoid on the other. The reduction of ectopterygoid has been recorded in *Typhlopidae* and *Glauconidae.* The ectopterygoid forms the base of the suborbital fossa. Zangerl (1944) recorded the abortion of this fenestra suborbitalis in Amphisbaenids on the ground that the palatine, ectopterygoid and pterygoid are suturally united. The ectopterygoid (Fig. 2) supports the maxilla rigidly. It does not form the pterygo-transpalatine projection at a place where it meets the pterygoid. The prevomers (Fig. 2) are devoid of the vomerine teeth. They are fused so as to leave no place for the formation of the prevomerine vacuity. The whole palatal apparatus is movably articulated with the skull, unlike those of a few burrowing lizards. The palatal vacuities though reduced due to the compactness of the bones, nevertheless, are more distinct from those of the temporal space.

The Upper Jaw :

The upper jaw has a single premaxilla and a paired maxilla. The azygous premaxilla forms the anteriormost region of the snout of the skull, while the maxillae bounds the snout laterally (Fig. 2). Each maxilla has a prominent jugal at the hind end.

The premaxilla :

The premaxilla or the intermaxillary is a solitary unpaired bone (Fig. 2). In Amphisbaenidae too Zangerl (1944) noticed an azygous premaxilla. The premaxilla is connected with the maxilla by ligamentous connections in Ophidia ; but in *Ophisaurus*, the premaxilla is fully ossified with the maxilla. The premaxilla has no teeth. Dorsally the premaxilla sends the processus nasalis to separate the nasals, and ventrally it forms a distinct but short and hard palatal vomero-maxillary process between the vomer and the maxilla, fused with the maxillo-palatine apparatus.

Adjacent to the premaxilla on either sides are the maxilla, to the summit of which are ankylosed a number of backwardly directed teeth. The maxillary teeth are pleurodont and sharply pointed as those of *A. fragilis*. Their backward projection towards the orbit is a peculiar feature. Among Amphisbaenids, Zangerl (1944) and Gervais (1853) noted acrodont dentition in *Trogonophus* and allied genera. The maxillae are fully supported at their ventro-posterior aspect by the transpalatines which serve to connect them with the pterygoids (Fig. 2).

The jugal :

The jugal (Fig. 3) is lodged at the posterior margin of the maxilla. It sends upwards a temporal process which in collaboration with the orbital process of the postorbital closes the orbit behind and thereby does not permit the continuity of the orbit and the neighbouring temporal space as in many lizards and most of the snakes. It is interesting to note that Zangerl (1944) recorded the total absence of the jugal in Amphisbaenians which is, however, distinct in *Opisaurus*.

The Suspensorium :

Ophisaurus has a unique suspensorial apparatus of the lower jaw, which approaches those of *Lacertilia* on the one side and of the *Ilysidae* and *Xenopeltis* (Ophidia) on the other. The articulation of the quadrate with the skull is through the squamosal which is protruding from the parietal and ranks next to it in the quadrate articulation of the skull. Some authors such as Gadow (1923) homologise the squamosal bone with the supratemporal bone of the temporal region of other lizards. In the absence of the supratemporal in this species of limbless lizard Gadow's homology may be accepted. The quadrate bone is mostly immovable in the allied families of limbless lizards composed of the degraded forms of various descent as mentioned by Gadow (1923). However, in *Ophisaurus*

the quadrate is highly movable for it is loosely connected at both the ends. This movement on the part of the quadrate due to the fibrous connections at both the heads, attributes it a streptostylic feature.

The quadrate :

The quadrate is a stout and cylindrical bone. It is vertically placed at the posterior end of the skull. It contains a dorsal and a ventral head and a straight body.

The dorsal head :

The dorsal head or the condylus cephalicus of the quadrate (Fig. 3) is attached ligamentously with the fused mass of the occipital ring of the cranium as well as with the squamosal with which it is loosely attached. The horizontally placed squamosal with its weak connection with the parietal gives additional support to the streptostylism.

The ventral head:

The ventral head or the condylus mandibularis (Fig. 3) of the quadrate has a double condylar facet for the articulation with the fovea articularis of the ramus mandibularis. This connection of the mandible with the quadrate is very feeble, adding to the movability of the jaw apparatus.

The quadrate body :

The body of the quadrate lies between the dorsal and the ventral heads of the quadrate. It is simple and straight part without any backward or forward projections or elevations with no groove in it for the tympanic attachment. The absence of the tympanum does not alter the position of the columella auris.

The Lower Jaw :

The mandible has two powerful ramii joined together not suturally but ligamentously at the symphysis. Each ramus mandibularis comprises four bones, the splenial being absent. The angularis is fused with the articular. The coronoid is prominent while the dentary has pleurodont dentition. Thus there are four bones in a mandible. Zangerl (1944) made out clear outlines of dentary, suprangular, coronoid and angular in Amphisbaenids.

The articular :

The articular (Fig. 5) is a small bone at the hinder margin of the ramus. A short arm lying behind is the postarticular or the processus

retro-articularis. The fovea articularis is a small foramena dorsally over the articular giving it a befitting modification for the condylus mandibularis of the quadrate which sinks into it.

The angular :

The angular is not a separate bone but is fused with the articular as in *Amphisbaenidae* and *Annellidae.*

The suprangular :

Facing the fovea articularis is the suprangular or surangular bone (Fig. 5) in between the coronoid and the articular (plus angularis). At this same level, but on the inner posterior aspect of the ramus, there is a short elleptical space called the fossa Meckelli (Fig 6)

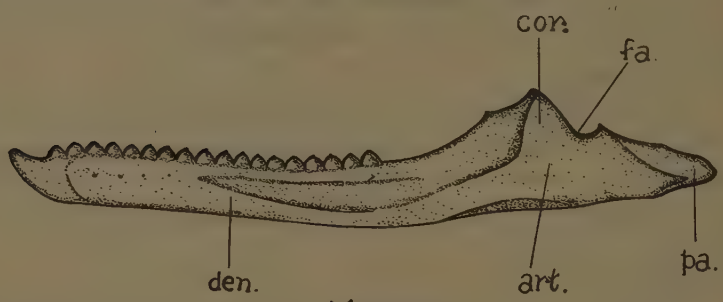

FIG. 5.

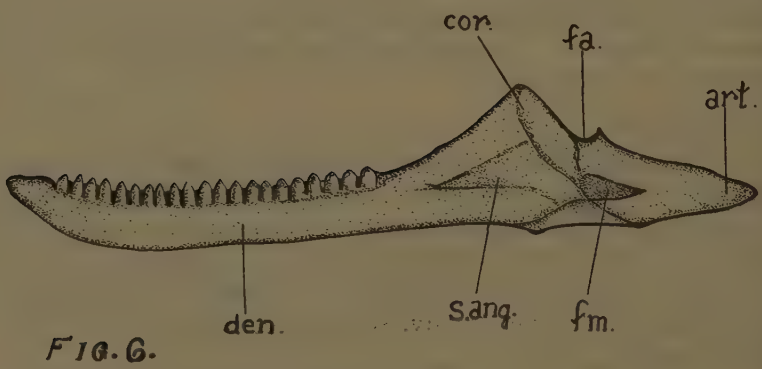

FIG. 6.

Fig. 5—Lower jaw (outer aspect) of *Ophisaurus gracilis.*
Fig. 6—Lower jaw (inner aspect) of *Ophisaurus gracilis.*
art. articular; cor. coronoid; den. dentary; fa. fovea articularis; fm. fossa Meckelli; pa post articular; s.ang. suprangular.

The coronoid :

The coronoid (Fig. 5) is a very distinct triangular bone unlike that of snakes. It resembles a nodule shaped coronoid of *Ilysiidae*. There is however, no processus messeterious of the coronoid at the cephalic region of it as seen in lizards. It (coronoid) simply touches the pterygo-transpalatine junction when the jaws meet the rest of the skull.

The splenial:

The splenial, which is generally a splint like bone and visible ventrally, has not been observed in *Ophisaurus* as also in *A. stellio*.

The dentary :

The major part of the lower jaw is occupied by the dentary (Fig. 6). A special ridge of the dentary bears pleurodont teeth which undergoes successive replacement. There is no groove below this dentary ridge for marking the sulcus cartilaginous Meckelli representing the distal end of the Meckel's cartilage.

The Kinetism:

On the basis of articulation of the quadrate, Stannius (1856) classified skull movements into streptostylism and Monimostylism. Versluys (1936) however, disproved Stannius's claim by citing a few ancestral forms where skull movements occur even when the quadrate was fixed. The movement of the quadrate has nothing to do with the various skull movements which he (Versluys) described collectively under the head kinetism.

The loose articulation of the quadrate with the skull and the mandible in *O. gracilis* fully endorses Stannius' (1856) streptostylism. Allied to Stannius' streptostylism is Versluys's (1936) kinetism. It is interesting to note that the palatal part of the skull bears the ligamentous connections at the various junctions between its elements. The basisphenoid is not movably articulated with the pterygoid in the absence of the basipterygoid. The quadrate part of the pterygoid in its turn approaches the inner ventral aspect of the movable quadrate. These conditions along with the curvature of the skull roof in front of the orbit, the latero-ventral extension of the parietal, the loose attachment of the bones of the palate, the massive development of the pro-otic and the supraoccipital without any ligamentous connections with the neighbouring bones and above all the absence of the cartilaginous cum membranous inter-orbital septum favour *mesokinetism* in the skull. The septo-maxilla and nasal enable a more favourably kinetic movement of the snout.

Discussion

This study reveals that the skull and the temporal region of *Ophisaurus* have a curious admixture of features common to both, lizards (Skinks and Amphisbaenians) and snakes (*Typhlops*, *Dasypeltes*, etc.). The cranial bones are solidly united as a rigid whole. The orbito-temporal region offers an a impressive illustration of convergent retrogressive evolution as far as the reduction in the temporal space (as in snakes) and the retention of the complete set of the circumorbital series (as in lizards) are concerned. The downgrowth of the frontals and parietals dispense with interorbital septum as in snakes and few other lizards *Gekkos* and *Varanids*; but it causes no disturbance to the *tropotrabic* arrangement of the skull (as in lizards). The parietals are paired and separate structures as in *Typhlops* and *Hemidactylus* (Mahendra, 1936 and 1948). As in snakes the cranium is perfectly solid. No fusion between the pro-otic, the supraoccipital and exoccipital occurs as in *Typhlops* and a few burrowing lizards, on the other hand the enlargement of the pro-otic and the supraoccipital eliminates the post-temporal fossa whereas the auditory capsule is degenerated (a scincidean character). The paired frontals, parietals and nasals and the absence of the parasphenoid as well as the basipterygoids are ophidian features. The obliteration of the upper temporal fossa by the postfronto-squamosal arcade and other dermal bones such as the parietal is an important difference, hitherto, unrecorded in saurians as well as in ophidians.

The contribution of the pre and the post-frontals in forming the supraorbital arch and the bony orbito-temporal processes of the post latero-frontal and jugal; a separate jugal and ectopterygoid together with the fused bones of the mandible, a peculiar jaw suspensorium, streptostylic quadrate and shifting up of the skull movements anteriorly towards the snout (mesokinetic) as well as the mobile palatal apparatus as in snakes confirm the view that *Ophisaurus* is in a transitory phase of phylogeny. Thus it may well be said that lizards and snakes are closely related and a similarity between them, more particularly between limbless lizards and snakes, is a very natural consequence. Incidentally, it should be mentioned, that Mcdowell and Bogert (1954) on certain osteological data have raised doubts regarding the systematic position of *Typhlops* under Ophidia and have suggested its removal from that group. George and Shah (1956) on the study of the lungs in reptiles have supported the in-

clusion of *Typhlops* in Ophidia. A discussion on the exact systematic position of Typhlopidae is, however, beyond the scope of the present paper.

Summary

(1) All the four occipital elements are fused into a single bony occipital ring. Both the supraoccipital and the parietal are situated at the same level. The single occipital condyle originates from the basi-occipital.

(2) The parietals are paired and separate bones without inter-parietal foramen. The latero-ventral extensions of the parietal fuse with the pro-otic. The basisphenoid has no basipterygoid processes. The basisphenoidal rostrum represents the incorporation of the parasphenoid with it.

(3) The frontals are paried. The prefrontals are separate from one another. The postfrontal fuses with the postorbital.

(4) The nasals are paired. The processus nasalis of the azygous premaxilla serves to separate them.

(5) The pro-otic is spaciously developed without any distinction of its components. This together with the massive development of the supraoccipital helps in eliminating the post temporal fossa.

(6) The squamosal is a prominent bone. Its feeble articulation with the parietal and the quadrate makes it homologous with the supra-temporal bone of other saurians.

(7) The postfronto-squamosal, the supraorbital and the orbito-temporal arches are present.

(8) Both the jugal and postorbital send the temporal and orbital processes to close the orbit behind. Confluence between the orbit and the temporal space is, therefore, not possible. The jugal, the postorbital, the pre- and the post-frontals and a definite lacrymal are the important members of the circum-orbital series.

(9) The postfronto-squamosal arcade along with the well developed parietal obliterates the supratemporal vacuity. The only temporal fossa is the infra temporal vacuity which opens below.

(10) The palatines are completely and the pterygoids partially denticulated with the pleurodont teeth. The prevomers are paired but have no vomerine teeth nor a prevomerine vacuity. The whole of the palatal apparatus is movably articulated.

(11) The lower jaw is typically lacertilian. The splenial is absent. The angular is fused with the articular. Dentition is pleurodont. The coronoid is distinct while there is nothing to represent the Meckel's cartilage.

(12) The suspensorium is streptostylic and the skull is mesokinetic and tropotrabic.

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STUDIES ON THE STRUCTURE AND PHYSIOLOGY OF THE FLIGHT MUSCLES OF BIRDS

1. The Variations in the Structure of the *Pectoralis Major* Muscle of a Few Representative Types and Their Significance in the Respective Modes of Flight.

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WHILE considerable amount of work has been done on the morphology, histology and physiology of the mammalian skeletal muscle, comparatively little is known of these aspects of the avian muscle. The ability to fly is generally conceived as a characteristic feature of birds though some like the *Ratitae* are completely flightless and others like the domestic fowl are unable to fly beyond just a few feet from the ground. Among the flying ones again, there exists a variety of forms exhibiting different modes of flight. A bird like the golden plover could fly long distances at a stretch and the humming bird hover in the air for a considerable length of time, both expending a tremendous amount of energy in the process, while the kite could remain soaring for hours at high altitudes with apparently minimum effort and expenditure of energy.

That there is a high energy output in the flight muscles of birds during long and sustained activity was realised as far back as 1893 by Marey, who observed, " The chemical reactions leading to the production of energy, are excited and propagated more readily in the muscles of birds than in those of any other species of animals ". Among the flight muscles the *pectoralis major* in all flying birds by far is the most important, because, structurally and functionally it is most well developed and modified. Nair (1954) studied the relation between the weight of this muscle and that of the body in some birds and obtained the lowest values for the domestic fowl and duck which are non-flying birds. George and Jyoti (1955a) who studied the histological structure of teased out fibres of the *pectoralis major* muscle of some birds and a bat observed that in the pigeon, this muscle is made up of two types of fibres—one which is narrow containing in its sarcoplasm dense lipoid granules and numerous fat globules which

render the characteristic striations hardly visible and that these fat doplets become fewer when the muscle is subjected to sustained exercise. In the other type, which is broad and devoid of the dense lipoid inclusions in its clear sarcoplasm, the striations are clearly visible. The broad variety of fibres was found to be predominating in the kite whereas in the fowl only this variety was found to be present and the narrow variety alone in the bat. In another paper they (1955b) discussed the possible role of fat as fuel in birds for sustained muscular activity. Recently George and Scaria (1956) showed the presence of lipase in the skeletal muscle of vertebrates and discussed its possible role in the utilization of fat as energy fuel in sustained muscular activity. Lawrie (1952) stressed the importance of myoglobin in the bird breast muscle as an oxygen carrying system.

The recent studies cited above have shown that the breast muscle of birds is a complex morphologico-physiological system and a clearer and comprehensive understanding of it from the structural and fuctional points of view should be of considerable value. It was therefore thought desirable to conduct a series of investigations on these aspects of the avian flight muscles and the present one is the first in the series.

Material and Methods

The *pectoralis major* muscle of the following birds were studied.

1. The domestic fowl *(Gallus domesticus)*
2. The pariah kite *(Milvus migrans)*
3. The blue rock pigeon *(Columba livia)*
4. The green parakeet *(Psittacula kramari)*
5. The green bee-eater *(Merops orientalis)*

The above birds were selected as representing the different structural types of the *pectoralis major* muscle and consequently different modes of flight : the fowl for a non-flying bird, the kite for a soarer, the parakeet and pigeon for a flapping flier and the bee-eater for a versatile flier exhibiting the flapping, shooting and gliding types of flight.

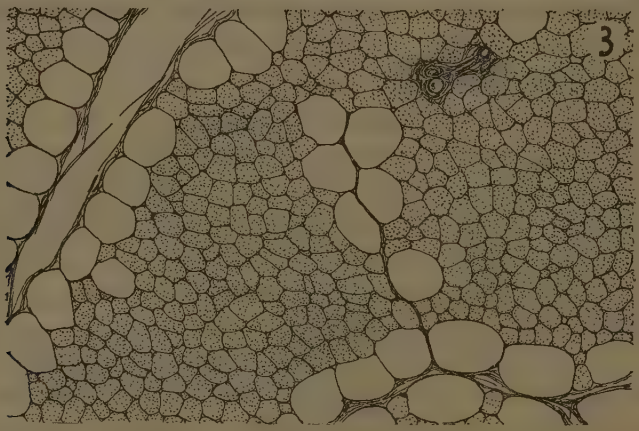
The birds were shot and within half an hour of killing small pieces of the *pectoralis major* were cut out and some were fixed in 10% neutral formalin, some in Zinker-formalin (Guyer, 1930) while the rest frozen fresh for sectioning. Paraffin sections of the pieces fixed in Zinker-formaline were cut at 8μ and stained with Mallory's phosphotungstic hematoxylin (Mallory, 1900) and observed under the microscope. Since

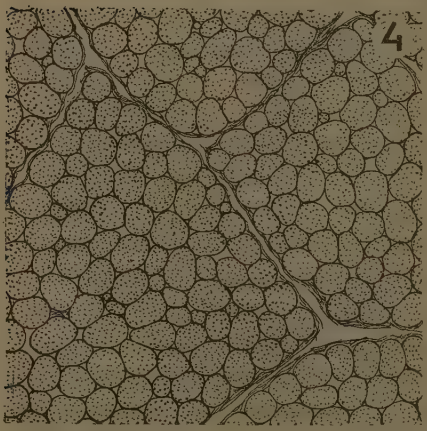
there was considerable amount of shrinkage, it was found more desirable to take the measurements of the fibres from the transverse sections of the fresh frozen material. Camera lucida sketches of the sections magnified to a thousand times were made and the diameter of the fibres was calculated from the measurements made from the sketches. In measuring the diameter of the fibres only the circular ones or the ones more or less circular were measured. The figures obtained are the average of several typical fibres from the muscle of three individual birds.

From the tissue fixed in 10% neutral formalin, individual muscle fibres were teased out by means of a couple of watchmaker forceps under a sterioscopic microscope and stained with Jackson's Aceto-Carbol Sudan III (Glick, 1949). These fibres were mounted in glycerine jelly for microscopical examination.

Observations and Results

On examining all the three preparations *viz.* the teased out fibres, the cross sections of the frozen tissue, microtome sections of the tissue fixed in Zinker-formalin, it was observed that the pigeon pectoralis consists of two types of fibres a broad type with clear sarcoplasm devoid of dense lipoid inclusions and a narrow one with dense lipoid granules and numerous fat droplets. The narrow variety constitutes about sixteen times the mumber of the other (George and Naik, 1957). The pectoralis of the fowl was found to consist of only broad fibres, so also of the kite, while that of the parakeet the narrow ones only. In the bee-eater pectoralis, however, the fibres are broader than those of the kite but with considerable lipoid inclusions. In the sections of fresh frozen muscle of the pigeon it is seen that the narrow fibres are red in colour and the broad ones white, the latter being more than twice broader in diameter than the former. The broad, white fibres circular in cross section are confined to the periphery of the muscle and also of the fasciculus within the muscle. On the other hand the red, narrow ones which are much more numerous are closely packed in the interior of the fasciculus. Often, a few white fibres too, are found in the interior of the fasciculus more or less in the middle. It also appears that the formation of a new fasciculus by the splitting of the parent one takes place in between two inner broad fibres (fig. 3) so that when the daughter fasciculi are formed they too will be lined by the broad ones on the periphery. All the fibres of the muscle in kite and bee-eater, are red and perfectly circular in cross section and





Camera lucida sketches of fresh, untreated transverse sections of the *pectoralis major* mus
of the domestic fowl (fig. 1), kite (fig. 2), pigeon (fig. 3), bee-eater (fig. 4) and parakeet (fig.
(Red fibres are shown dotted) × 103

loosely packed within the fasciculus (figs. 2 & 4). Those of the parakeet pectoralis too are red but unlike those of the kite and bee-eater are very much narrower in diameter and closely packed within the fasciculus (fig. 5). All the fibres of the fowl pectoralis, on the other hand are white, much broader and most of them polygonal in cross section, and a few more or less circular (fig. 1).

Table 1

No.	Bird		Nature of the fibres	Average diameter μ
1.	Fowl		White With clear sarcoplasm Polygonal in c. s.	64
2.	Kite		Red Clear sarcoplasm Circular in c. s., loosely packed	43
3.	Pigeon	1.	Red Fat loaded Closely packed in the interior of the fasciculi	33
		2.	White With clear sarcoplasm Bordering the fasciculi	70
4.	Merops		Red Fat loaded Circular in c. s., loosely packed	50
5.	Parakeet		Red Fat loaded Closely packed	30

The lipoid inclusions of the fibres are best observed in the teased out entire fibres stained with Jackson's stain. The fibres of the parakeet pectoralis and the narrow fibres of that of the pigeon both of which are red fibres, are loaded with dense lipoid granules and fat droplets. These

inclusions are fewer in those of the bee-eater which are also red fibres. On the other hand those of the kite, fowl and the white ones of the pigeon pectoralis have clear sarcoplasm devoid of dense lipoid inclusions. In the kite pectoralis however, considerable amount of lipoid matter was noticed in the connective tissue enveloping the fibres as well as the fasciculi.

The diameter and certain general features regarding the structure of the fibres in the *pectoralis major* muscle of the different birds studied are given in a tabular form (table 1).

Discussion

Muscular tissue in general exhibits a wide range of morphological and biochemical variations. That the homologous muscles of animals belonging to the same group may show great variations in microscopical structure is seen in the flight muscles of insects (Tiegs, 1955). The muscles of vertebrates are by no means an exception to this. It is well known to physiologists that different muscles react differently to the same chemical. Such differential behaviour in many cases could be attributed to the difference in structure and function of the muscle. That such differences in microscopical structure in the same muscle in different animals do occur is well indicated in the present study.

George and Jyoti (1955a) showed that the bat breast muscle consists of narrow fibres with dense lipoid inclusions unlike those of its leg which are broad and with clear sarcoplasm and hardly few small fat droplets. Kenny and Glenn Richards in a recent publication (1955) noted the presence of narrow fibres (75μ in diameter) in the flight muscle, and broad ones (350-400μ in diameter) in the leg muscle of the giant water bug, *Lethoceros americanus*. The occurrence of the narrow type of fibres in the breast muscles of flying animals like the bird, bat and insect is therefore an interesting case of convergence. It also suggests a physiological correlation with the morphological differences seen in the flight and the leg muscles of one species as well as in the flight muscles of different flying animals. It has been shown that the pigeon *pectoralis major* consists of two types of fibres, one is the broad variety with clear sarcoplasm and the other narrow type which is about sixteen times the number of the former. George and Naik (unpublished, 1957) have also found that the narrow variety in the pigeon pectoralis is heavily loaded with fat while the broad variety with glycogen. In the light of the recent observations of George and Jyoti (1955a and 1955b) and George and Naik (unpublished, 1957)

and of George and Scaria (1956) showing that there is about eight times greater lipase activity in the pectoralis of the pigeon than in that of the fowl, it could be concluded that the narrow variety of fibres with the characteristic lipoid inclusions appears to be specially adapted for long and sustained activity in which fat is the chief fuel, and the broad variety with clear sarcoplasm practically devoid of lipoid inclusions for quick and faster action in which glycogen forms the chief fuel. The *pectoralis major* of the pigeon therefore could be considered as composed of two components, one of the broad glycogen-loaded variety and the other narrow fat-loaded variety of muscle fibres. The latter ones are distinctly different from the former in colour, size, structure, in chemical composition and in all probability in their physiology as well. So, for the two muscle components of the pigeon *pectoralis major* one should expect two entirely different modes of action based on their respective structure and physiology.

The fibres of the kite *pectoralis major* are of the broad variety like those of the fowl but for the fact that the kite fibres are red in colour and the fowl ones white. The fibres of the kite, like those of the pigeon, parakeet and bee-eater which are all flying birds, are circular in cross section, whereas, those of the fowl which is a nonflier, are polygonal in cross section. The polygonal shape is intimately related to the nonflying habit of the fowl, because, in inactivity there is a certain amount of stability that is established, with the result that the fibres tend to acquire more connective tissue to become fixed and thickened at places now distinguished as the corners of the polygonum. This could not happen in the case of the constantly moving fibres of other birds and they therefore retain their original circular pattern in cross section. The fibres of the bee-eater, though broad in diameter compared to those of the kite, have considerable amount of lipoid inclusions, a feature, hitherto not noticed in the broad fibre of others. It is therefore obvious that the method of describing the *pectoralis major* muscle of different birds on the basis of the diameter of the fibres alone is unsatisfactory. Other factors must also be taken into account. The method of distinguishing the *pectoralis major* of one bird from that of another on the basis of its colour (or the colour of the individual fibres) is equally unsatisfactory because this muscle in the pigeon is red but on microscopical examination contains white fibres too. Again the distinction on the basis of colour alone namely red, pale and white is not sufficiently sharp so as to deserve the dignity of a scientific method of demarcation. Nevertheless, the method of distinguishing the breast mus-

cles of birds as red and white when certain other factors are also taken into account, becomes extremely useful. The red fibres in possessing the oxygen carrying system have a definite advantage over the white ones in sustained muscular activity. In this connection the surface area of the fibres is an important factor in effecting the gaseous exchange more efficient. Since greater the number of fibres per unit area, the greater would be the surface area, naturally lesser should be the size of the fibres. So the diameter of the fibres should be an index of the surface area. Among red muscles, therefore the narrow fibres of the pigeon and parakeet should be a very efficient oxygen carrying system. Since a good supply of oxygen is essential for the utilization of fat to be used for energy, these narrow fibres should be a system well suited for the utilization of fat as fuel in sustained muscular activity. On the other hand, the broad, white fibres of the pigeon breast muscle as well as those of the fowl would have to depend more on the glycogen store for energy and such energy could be made available under anaerobic conditions.

The task of differentiating the breast muscles of different birds on the basis of certain structural and physiological characteristics is, however, a difficult one. Nevertheless, if one takes into account, the colour, diameter, inclusions, and the nature of the fuel store of the fibres together with the mode of action of the muscle as a whole in relation to the particular mode of flight of the bird concerned, it is possible to develop a tentative plan. Among the birds studied, the fowl is a nonflying type, its breast muscle is white, fibres are broad, practically without lipoid inclusions and glycogen loaded. Among the red muscles, kite breast muscle consists of broad fibres which are devoid of dense lipoid inclusions and chiefly glycogen-loaded. So the kite muscle is not suitable for sustained activity as is exhibited by a flapping bird like the parakeet in which the fibres of this muscle are red, narrow, with dense lipoid inclusions and fat-loaded. The kite therefore is an elegant soarer. In the versatile flier, bee-eater, the fibres are red, broad, with lipoid inclusions and fat-loaded. The pigeon breast muscle presents a unique feature in having both the types of fibres. The pigeon is essentially a flapping bird at times could perform a shooting action perhaps with the aid of the broad variety of fibres. The exact role of the broad variety of fibres in flight is, however, doubtful but it is hoped that further investigations would throw some new light.

Examining the data obtained in the present study it is possible to

visualise, as follows, the possible lines of modifications in the evolution of the *pectoralis major* muscle of carinaceous birds and also account for the differences in the respective modes of flight.

—Red Fibres
- Fat-loaded—
  - Narrow, *e.g.* Parakeet—Flapping type of flier
  - Broad, *e.g.* Bee-eater—Versatile type of flier
- Glycogen-loaded Broad, *e.g.* Kite—Soaring type of flier

—Mixed fibres (Red and white)
- Fat-loaded Narrow
- Glycogen-loaded Broad

e.g. Pigeon —Flapping type of flier

—White fibres—Glycogen-loaded Broad *e.g.* Fowl—Non-flying type

We are fully aware of the fact that it is not safe to venture generalisations of this nature basing conclusions on observations made on a few types. Nevertheless, we feel it useful and desirable to put forth these ideas as a prelude to the studies we propose to conduct in the series. Our knowledge regarding the origin and evolution of flight in birds is far from clear and we have a classification of birds which is far from satisfactory in comparison with other groups and based on comparatively minor morphological features while the major strides in the evolution of this group have been physiological. This is also evident from the fact that the palaeontological studies, which have contributed so much towards our understanding of the main tracks of evolution have comparatively done little with regard to birds. This series of studies is therefore an attempt to peep into the vast corners of the subject of avian evolution.

Summary

1. The structure of the *pectoralis major* muscle of a few birds exhibiting different modes of flight is studied.

2. The significance of the colour, size, distribution and lipoid inclusions of the fibres of this muscle, in the different modes of flight is discussed.

3. The possible lines of fibrillar modification in the evolution of the *pectoralis major* muscle of carinaceous birds in relation to the respective modes of flight are envisaged.

Acknowledgment

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ON THE PRESENCE OF SPECIALISED CONNECTING (CONDUCTING) TISSUE IN THE HEART OF THE INDIAN BLUE ROCK-PIGEON *COLUMBA LIVIA INTERMEDIA* STRICK

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[1 Plate]

KEITH and Mackenzie (1910) and Mackenzie and Robertson (1910) denied the existence of sinuatrial and atrioventricular nodes in birds. Davies (1930) while correlating the peculiar and intricate details of the Purkinje system (Purkinje, 1845) with the functional requirements of the avian heart pointed out that in birds a sinuatrial node, an atrioventricular node, an atrioventricular bundle and multiple muscular connexions of Kent (Kent, 1893) are present to initiate and transmit the cardiac rhythm of contraction. According to Davies (1930) the atrioventricular conducting tissue of the bird's heart presents an arrangement which is intermediate between that of fish and reptile on one hand and that of the mammal on the other. However, Davies and Francis (1946) showed that the specialized tissue of the avian and the mammalian heart is neomorphic in nature and is not a remnant of that of lower vertebrates (fish, amphibia and reptile).

Adams (1937) studied the heart of *Apteryx australis* and *Megadyptus antipodum* and observed that sinuatrial region of the bird's heart is essentially similar to that of reptiles. He also pointed out that the incorporation of the sinus muscultaure into that of the atrium in birds presents an intermediate stage between reptiles and mammals. According to Adams (1937) in most birds there is a clear resemblance to the reptilian condition than to mammalian. Adams (1937) was also able to locate a nodal area and an atrioventricular bundle (bundle of His, 1893) in *Apteryx* and *Megadyptus*.

De Mayer (1952) denying the existence of a specialized impulse initiating and conducting tissue in avian heart pointed out that sinus portion of the conducting tissue disappears in the heart of the adult fowl

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and that an atrioventricular bundle is either reduced or absent in the heart of sparrow. Prakash (1956) maintained that sinuatrial node, atrioventricular node, atrioventricular bundle and Purkinje fibres are present in the heart of the common Indian fowl for initiating and conducting cardiac stimulus of contraction. This investigator, however, did not refer to multiple muscular atrioventricular connexions of Kent which Davies (1930) regarded of paramount importance for a quick transmission of the atrial stimulus of contraction to ventricles in correlation with the rapid rate of heart beat of birds. Prakash (1953, 1954, 1956, 1957) in a series of papers maintains that the impulse initiating and conducting tissue of birds and mammals is not a neomorphic development but has evolved from that of cold blooded vertebrates.

The foregoing review indicates that studies on the specialized conducting (connecting) tissue of the bird's heart have been very few and that investigators are not unanimous in their view about the presence and disposition of this tissue in birds. In the present investigation the heart of Indian Blue rock pigeon, *Columba livia intermedia* has been studied with special reference to the conducting (connecting) tissue.

Material and Methods

For the present investigation about a dozen specimens of well fed and healthy Indian Blue rock pigeon, *Columba livia intermedia* were procured.

The hearts after being removed from chloroformed pigeons, were fixed in Bouin's picroformal. Serial sagittal and frontal sections 8 to 10 micra thick were cut of paraffin embedded blocks of heart. Sections were stained in acid fuchsin and examined under Reichert's fibroscope. Photomicrographs were also taken with the aid of this apparatus.

Observations

The heart has four chambers *viz.*, two atria and two ventricles. Sinus venosus is absent.

Sinuatrial node : At the opening of the left precaval vein into the right atrium, is present a well defined tissue of multinucleated cells and long narrow, slender fibres which have taken a deep stain with acid fuchsin, and appear quite distinct from the surrounding tissue. The special histological nature and the position of this tissue warrants its (Fig. 1) identification as the sinuatrial node which is held to be responsible for initiating

the cardiac rhythm of contraction in birds. The fibres of the node appear as bundles and present an alveolar arrangement when observed in transverse sections. This node appears as an extensive, well defined and conspicuous structure in the heart of *Columba* and its component fibres are interwoven to form cells each of which shows a clear central space, and a number of peripherally placed myofibrillae. These are the characteristics of Purkinje fibres and therefore, minute anatomy of sinuatrial node as studied in the present investigation points out that Purkinje fibres are present inside this node. The presence of a nodal artery very near to the node shows that the tissue has been correctly identified as sinuatrial node. The specialized fibres of sinuatrial node enter deep into the interatrial septum and extend into that portion of the atrial wall which lies in contact with the cephalic portion of the interatrial septum. Purkinje fibres were not seen in the interatrial septum but instead it was observed that fibres of the sinuatrial node freely communicate with the muscular components of interatrial septum. Groups of Purkinje fibres were observed at certain places in the wall of the two atria but any continuity between these fibres and those of the sinuatrial node was not traceable.

Atrioventricular node: This node is present as a meshwork of loosely arranged specialized fibres and cells in the caudal part of the interatrial septum. The fibres of this node are long, narrow and are more loosely arranged than those of the sinuatrial node. The triangular shaped atrioventricular node is well demarcated from the surrounding tissue because it is enclosed in a compactly arranged layer of fibres. The apex of this triangular atrioventricular node is directed towards the cephalic portion of the right atrium and on either side of it are present two vessels to be referred to as nodal arteries. The nodal artery present on the right side of the atrioventricular node is larger than that present on its left side. The association of the atrioventricular node with two nodal arteries is a special hitherto unrecorded feature of the heart of *Columba*. Closely packed cells with one nucleus and a clear perinuclear space have been observed inside the atrioventricular node. One important feature observed for this node in *Columba* is that its cells are arranged on the periphery and in the centre there is only a fibrous mass. The atrioventricular node though situated in the caudal portion of the interatrial septum (Fig. 2) rather extends more towards the right side so that its major portion comes to lie in the subendocardial region of the right atrium. This node freely communicates with the atrial fibres lying all round it through the fibres

which enclose it all round. Ventrally and caudally the node is continued into an atrioventricular bundle (His, 1893).

Atrioventricular bundle: The atrioventricular bundle is also composed of Purkinje fibres and cells grouped together and enclosed in a definite fibrous sheath so as to appear in the form of a conspicuous, well defined and demarcated structure in serial sections of the heart (Figs. 3, 4 & 5). The fibres of the atrioventricular node extend downwards along the right lateral border of the interatrial and the interventricular septa to become continuous with the muscular components of the atrioventricular bundle which climbs up so as to be present in the cranial portion of the interventricular septum. The bundle is present just beneath the node.

It is interesting to note in this connection that a continuous band of Purkinje fibres connecting the atrioventricular node and the bundle is present and that too on the right side only. Along the left lateral border of interventricular septum no Purkinje fibres interposed between the node and the bundle but on this side the connection is established through ordinary but innumerable cardiac fibres.

As generally observed in the bird's heart (Davies, 1930 and Prakash, 1956) the atrioventricular bundle branches caudally to form a right and a left limb which descend down respectively on right and left sides of the interventricular septum. The right branch extends along the right lateral border and the left along the left lateral border of the interventricular septum. From the bundle also extends a recurrent branch (Davies 1939) which enters the base of the right muscular valve. From the left limb of the bundle Purkinje fibres extend towards the aortic arch to appear in sections of the heart, in the form of an oval bundle, at its base.

Multiple muscular atrioventricular connexions: The ordinary innumerable fibres connecting the atrioventricular node with the atrioventricular bundle along the left lateral side of the interventricular septum, are present (Figs. 3, 4 and 6) in cross sections of the heart in the form of an oval bundle in close association with and very near to the bundle of His (His, 1893). These multiple muscle fibres do not exhibit any of the histological details observed for Purkinje fibres (Figs. 3 and 4). They are not interlaced with each other and do not show cells having oval shaped and deeply stained nuclei surrounded by a clear space of cytoplasm. They freely communicate cranially with the atrioventricular node lying in

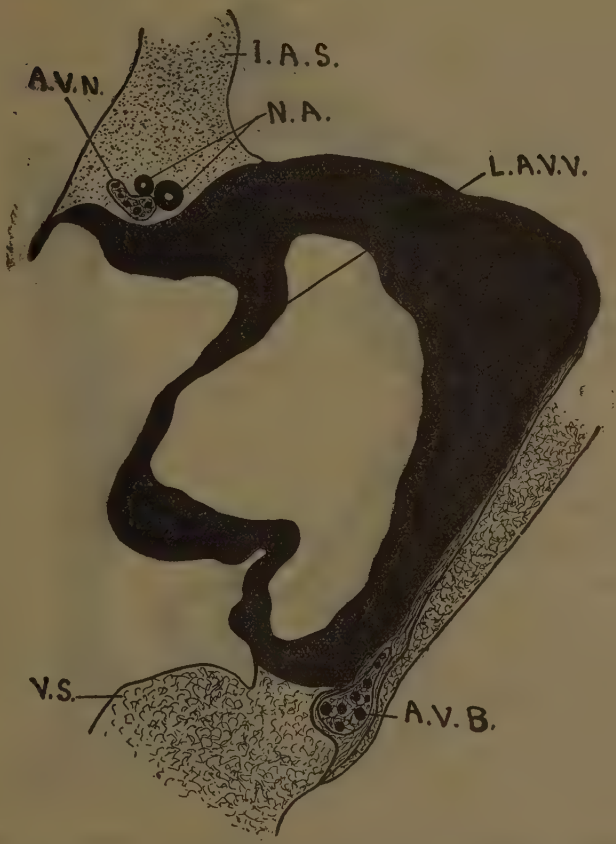

Fig. 5. Diagram of a section of heart showing the atrioventricular node and atrioventricular bundle.

A. V. B. Atrioventricular bundle; A. V. N. Atrioventricular node;
I. A. S. Interatrial septum; L. A. V. V. Left atrioventricular valve;
N. A. Nodal artery; V. S. Ventricular septum.

the interatrial septum, and caudally with the atrioventricular bundle lying in the interventricular septum, and thus connect the former with the latter.

Discussion

The present investigation confirms the presence of histologically specialised impulse initiating and conducting structures in the form of sinuatrial node, atrioventricular node and atrioventricular bundle in the heart of birds. The structures referred to above are formed of and connect-

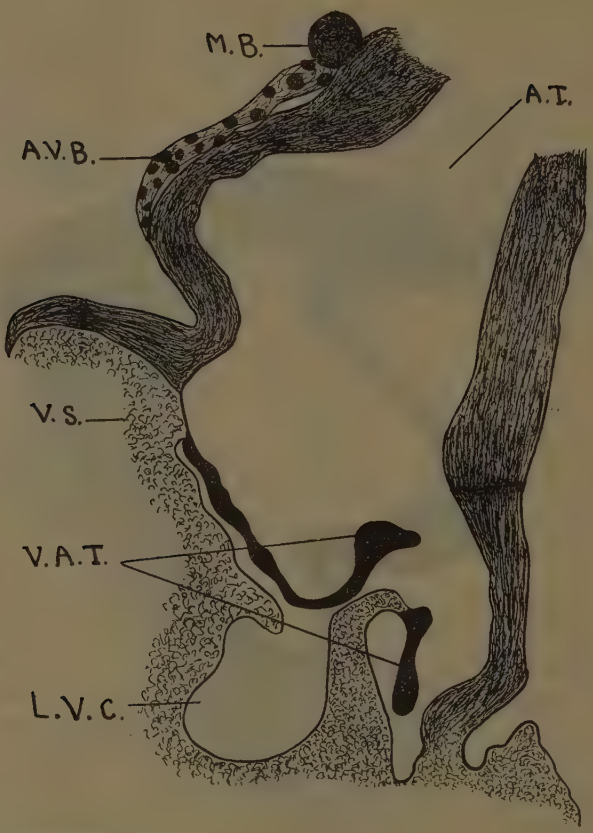

Fig. 6. Diagram of a section of heart showing the atrioventricular bundle and bundle of accessory connexions.

A. T. Aortic trunk; L. V. C. Left ventricular cavity; M. B. Muscular bundle of accessory atrio ventricular connexions; V. A. T. Valves of aortic trunk; (Rest same as in fig. 5)

ed with each other through Purkinje fibres and therefore, Purkinje fibres seem to be important constitutents of the cardiac conducting system (Prakash 1954 and 1956) of birds.

Prakash (1954) stated that "the sinuatrial node develops in relation with and consequent to the reduction of the sinus venosus". In the heart of the rat, *Rattus rattus rufecens* (Prakash, 1954) sinuatrial node is not present but instead a well developed sinus venosus is described. In the heart of albino rats (Prakash, 1957) with the total disappearance of sinus

venosus a well developed and an extensive sinuatrial node is present. In the heart of the common Indian fowl (Prakash, 1956) a reduced sinus venosus has been described and it is stated that " With the reduction of the sinus venosus the nodal area and the sinuatrial node which are present on either side of this chamber will get united to form an extensive sinuatrial node extending from the base of right sinuatrial valve over to the cephalic and dorsal side of the right atrium". In the heart of *Columba* sinus venosus is completely incorporated into the right atrium and a sinuatrial node is present.

It has been observed that the Purkinje fibres do not connect the sinuatrial node with the atrioventricular node and that only ordinary cardiac fibres establish a continuity between these two nodes to transmit the cardiac stimulus of contraction from the former to the latter.

Rhythmic impulses received at the atrioventricular node would be conveyed to the atrioventricular bundle through two pathways as the node and the bundle are connected to each other through Purkinje fibres along the right side and through multiple muscle fibres along the left.

The presence of two pathways, one formed of Purkinje fibres and the other of ordinary fibres, is a point of considerable interest. It shows that the ordinary as well as the specialised fibres form pathways for the conduction of the cardiac rhythm. It also shows that correlated with the rapid rate of heart beat of birds two pathways for a quick transmission of the atrial stimulus of contraction to the ventricles are present. Multiple muscular connexions which connect the atrioventricular node, lying in the caudal portion of interatrial septum, with the atrioventricular bundle, lying in the cephalic portion of interventricular septum, cannot be regarded as accessory to the bundle of His. There presence in no way would supplement the activity of the atrioventricular bundle because their disposition shows that they would conduct the contraction stimulus from the atrioventricular node to the atrioventricular bundle and not directly from the node to the ventricles. Ofcourse, these fibres serve as additional connexion between the node and the bundle ; and as the node lies in the interatrial septum and the bundle in the interventricular septum these may be called as accessory atrioventricular connexions. However, the atrioventricular bundle remains of paramount importance so far as the conduction of contraction impulses to the ventricles is concerned. The accessory atrioventricular connexions as well as the Purkinje fibres would

bring the cardiac rhythm of contraction from the atrioventricular node to the atrioventricular bundle and it will be only this bundle which would transmit the impulses to the ventricles through its two limbs.

Many investigators (Davies, 1930; Kistin, 1949; Prakash, 1954) have regarded the multiple muscular atrioventricular connexions as "Accessory" to the bundle of His. The present investigation shows that they are not so and that their presence is essential in avian heart for a quick transmission of the contraction stimuli from the atrioventricular node to the bundle of His.

Summary

1. The structure and disposition of the tissue that connects the various chambers of the heart of the Indian blue rock pigeon *Columba livia intermedia* has been studied.

2. A sinu atrial node, an atrio ventricular node and an atrio ventricular bundle with its two limbs are present.

3. Purkinje fibres as well as typical cardiac fibres form pathways for the conduction of the cardiac rhythm of contraction.

4. Two pathways one of specialized fibres and the other of ordinary fibres have been observed to transmit the contraction stimulus from the atrio ventricular node to the atrio-ventricular bundle. As the node and the bundle lie in the interatrial septum and the interventricular septum respectively the ordinary innumerable fibres connecting the two structures have been referred to as Accessory atrioventricular muscular connexions.

5. Accessory atrioventricular connexions are essential in bird's heart for a quick transmission of the atrial stimulus of contraction to the ventricles.

6. It is held that the accessory atrioventricular muscular connexions do not aid the bundle of His and that it is the latter structure only which is responsible to distribute the stimulus of contraction to the ventricles.

Acknowledgment

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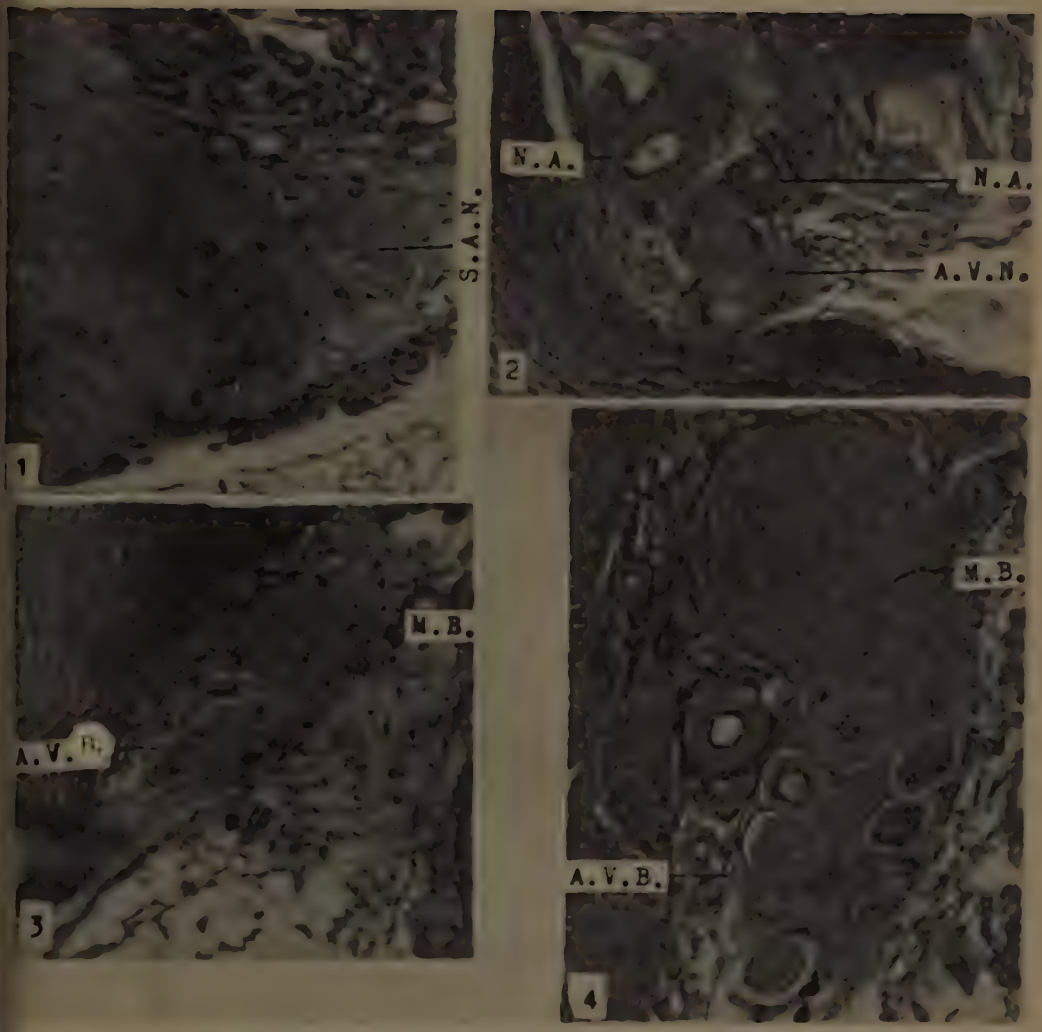

(Figures 1 to 4 are untouched photomicrographs of localised areas)

Fig. 1. Section through the sinuatrial node of the heart of *Columba*. X 225.

Fig. 2. Section through the atrioventricular junction of heart showing the atrioventricular node and the two nodal arteries. X 225.

Fig. 3. Section of the heart showing the atrioventricular bundle and the muscular bundle of accessory multiple muscular atrioventricular connections. X 225.

Fig. 4. Atrioventricular bundle and bundle of accessory connexions under high power. X 450.

A. V. B. Atrioventricular bundle; A. V. N. Atrioventricular node;

M. B. Muscular bundle of accessory atrioventricular connexions;

N. A., Nodal artery; S. A. N. Sinuatrial node.

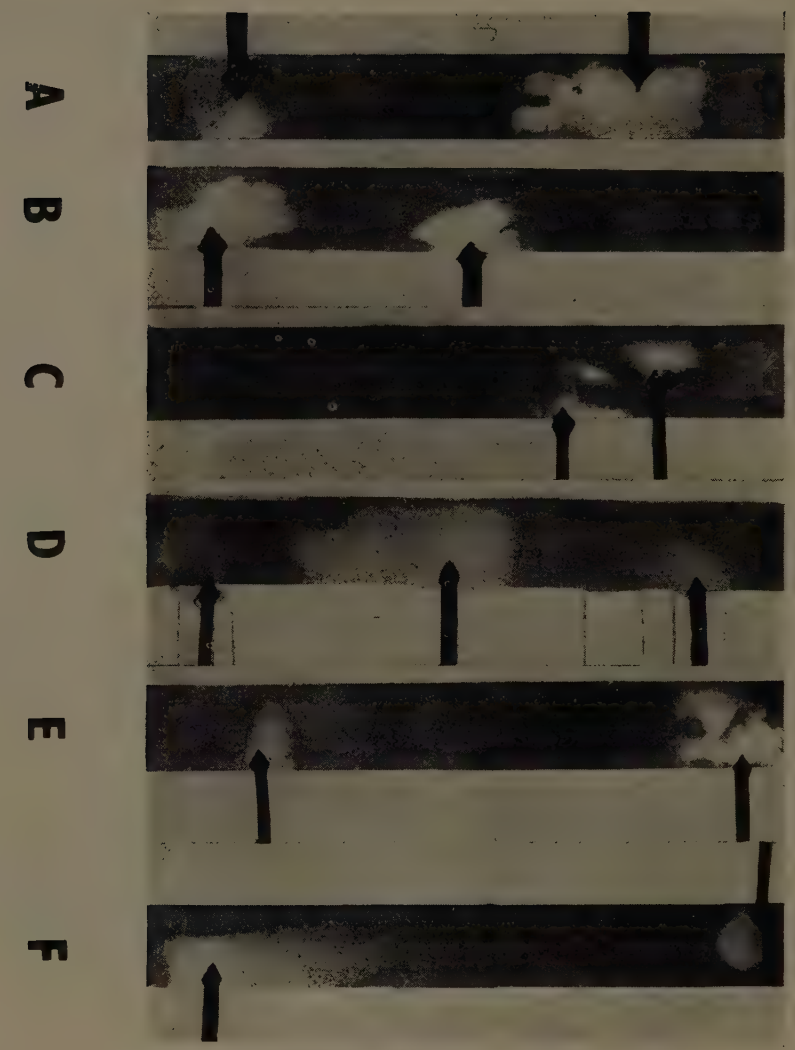

Fig. 1 Chromatogram of amylases of liver and intestine of Mullets.
Arrows indicate the position of the amylase fractions.
A & B *Mugil speiglari*—Liver and intestine amylases respectively.
C & D *M. dussumeri*—Liver and intestine amylases respectively.
E & F *M. cephalus*—Liver and intestine amylases respectively.

AMYLASES IN MULLETS

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[1 Plate]

The amylases in fishes which have been investigated so far have been studied mostly with reference to the optimum conditions required for their activity. Babkin (1927) studied the amylase in the intestinal mucous membrane of *Fundulus heteroclitus*. Vonk (1927) investigated the amylase in the pancreas of the carp, pike and dog fish. MacKay (1929) found a strong amylase in the duodenal mucous membrane of *Zoarces anguilaris*. Chesley (1934) investigated the amylases of a few marine fishes. Bayliss (1935) who studied the digestive enzymes of *Pleuronectes platessa* found amylases with identical optimum pH in the liver, intestine and gall bladder. Sarbahi (1951) investigated the distribution of amylases in the liver and intestine of gold fish and the pancreatic tissue of the large-mouth black bass. Of the Indian fishes the amylase of *Ophicephalus punctatus* was studied by Mahalanabis and Chauduri (1951). More recently Ghanekar *et al.* (1956) have made a detailed study of amylases of elasmobranchs with reference to the optimum pH and activity.

But in none of the investigations made in teleosts so far, has any attempt been made to determine whether the amylase studied was a single enzyme or a mixture of enzymes. The present study is a chromatographic investigation of the amylases of three species of mullets, *Mugil speiglari* (Bl. Kr.), *Mugil dussumeri* (C. V.) and *Mugil cephalus* (Linn).

Material and Methods

The amylases of the liver and intestine were studied in the three species. Glycerol extracts of these tissues were prepared separately and diluted with distilled water to a strength of 10%. Descending chromatograms were run on strips of Whatman filter paper No. 1 (35 cm × 4 cm) with 50% acetone as solvent, adopting the procedure described by Giri and Prasad (1951) in their investigation of the amylases of sweet potato and *Aspergillus niger*. The chromatograms were run for 15 to 18 hours. The paper strips were taken out and after marking the solvent front, were

allowed to dry at room temperature. The dried paper strips were then placed on agar (2%) - starch (1%) solution in a phosphate buffer of pH 6.4 to 6.8 in large petri dishes. The petri dishes were placed in an incubator at 34° C for 4 to 5 hours. The strips were then removed, and washed with N/100 iodine solution. The position of amylase fraction was recognised by clear white or violet spot on a blue background. The R*f* values of the spots were determined according to the procedure of Consden *et al.* (1944), — i. e. $Rf = \frac{\text{movement of the band}}{\text{movement of the advancing front of the liquid}}$. Table I shows the R*f* values of the spots.

Results

The amylase from the liver of *Mugil speiglari* separates into two fractions with different R*f* values of 0.31 and 0.77 respectively (Fig 1 A.). The amylase of *Mugil dussumeri* also separates into two fractions—one with R*f* value 0.22 and the other with R*f* of 0.33 (Fig. 1 C.). The amylase fractions of *Mugil cephalus*, which are also two, show R*f* values of 0.13 and 0.81 respectively (Fig 1 E). It will be observed that *M. dussumeri* and *M. speiglari* have nearly similar R*f* values for one of their amylase fractions (0.31 and 0.33), and likewise *M. speiglari* and *M. cephalus* resemble in the R*f* values of one of their liver amylase fractions.

The amylase of intestine of *M. speiglari* separates into two fractions with R*f* values of 0.43 and 0.80 respectively (Fig. 1 B) The amylase of *M. cephalus* also separates into two fractions but with R*f* of 0.10 and 0.84 (Fig. 1 F). The intestinal amylase of *M. dussumeri* on the other hand separates into three fractions with R*f* values of 0.32, 0 63 and 0.89 (Fig. 1 D). It will be observed, as in the case of liver amylases, there is some resemblance among the three species. For example, *M. speiglari* and *M. cephalus* have an enzyme with similar R*f* value (0.80 and 0.84).

Yet another feature of interest is that the two fractions of the liver amylase and the intestinal amylase of *M. cephalus* have almost the same R*f* values (0.13 and 0.10 ; 0.81 and 0.84). One of the fractions of the liver amylase of *M. dussumeri*, which has R*f* value of 0.33 has a counter part with R*f* = 0 32 in one of the intestinal amylase fractions.

TABLE 1

Rf values for fractions of amylases of liver and intestine of Mullets.

Name of the species	Rf values of amylase of liver	Rf values of amylase of intestine
Mugil speiglari (Bl. Kr.)	0.31 0.77	0.43 0.80
Mugil dussumeri (C. V.)	0.22 0.33	0.32 0.63 0.89
Mugil cephalus (Linn)	0.13 0.81	0.10 0.84

Conclusion

The fact that the amylases in each of the three species of mullets investigated separate into fractions with different Rf values would indicate that their amylases have different chemical composition. At the same time there is some similarity, though not identity in the Rf values of the amylase fractions of the three species. Within each species the amylases of the liver and intestine are not identical although they seem to be similar in *M. cephalus* as indicated by the Rf values. Apart from these similarities, and dissimilarities, the present investigation indicates that the amylases from the liver and intestine are not single enzymes but mixtures of enzymes, since they separate into fractions with different Rf values. The presence of a liver amylase in teleosts, which has been observed by Bayliss (1935) also, is probably due to the presence of pancreatic tissue embedded in liver.

Giri and Prasad (1951) have shown that the β amylase of sweet potato shows in the chromatogram two violet spots with Rf values of 0.53 and 0.61 respectively. One of the fractions of the intestinal amylase of *M. dussumeri* resembles in its violet colour and Rf value (of 0 63) the β amylase of sweet potato. Further, one of the fractions of the amylase of liver of *M. speciglari* gives a clear white spot with Rf value of 0.77. The amylase of *Aspergillus* has a similar fraction with Rf 0.77.

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THE EFFECT OF OSMOTIC PRESSURE ON THE GROWTH AND DEVELOPMENT OF *AEDES AEGYPTI* LARVAE

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AEDES aegypti has defied the attempts of many workers to rear it on a chemically defined diet. It has been possible to rear this species only if casein or yeast fractions were present in the diet. As the complete analysis of casein is known, there should have been no difficulty in replacing it with individual amino acids. Nevertheless, Golberg and De Meillon (1948) and many other workers were not able to substitute an amino acid mixture for casein. Singh (1955) and Singh and Brown (1957) also attempted to rear *A. aegypti* larvae on a chemically known diet, but the larvae did not grow beyond the first instar and died after a few days. This inhibition of growth in the first instar and death of the larvae could not be explained on the basis of some chemical deficiency or the direct toxicity of any dietary compound. The most evident factor in the diet which could have been changed was suspected to be the osmotic pressure of the diet medium, due to the presence of osmotically active amino acids. Therefore, the effect of osmotic pressure of the diet medium on the growth and development of *A. aegypti* was studied.

Material and Methods

To study the effect of osmotic pressure, the diet containing insoluble residue of yeast as a protein factor, glucose, inorganic salts, RNA, and vitamins, (thiamine, riboflavin, nicotinic acid, pyridoxine, pantothenic acid, choline chloride, folic acid) (Singh and Brown 1957), was taken as the basic diet. On this diet *A. aegypti* larvae can complete the whole life cycle in 9-10 days. To avoid any toxic effect on the growth by inorganic salts, in the first set of experiments, diets with osmotic activity from $\triangle$ 0.12°C. to $\triangle$ 0.98°C. or equivalent to 0.18 to 1.47 per cent NaCl, were obtained by changing concentration of glucose in the basic diet. The osmotic pressure of the diets was measured as freezing point depression by Backmann's cryoscopic method.

In the second set of experiments the diet used consisted of glucose,

inorganic salts, lipids (cholesterol, lecithin, cephalin), RNA, glutathione, vitamins (thiamine, riboflavin, nicotinic acid, pyridoxine, pantothenic acid, choline chloride, B_{Γ}, B_{12}) and amino acids (alanine, arginine, cystine, glycine, histidine, hydroxyproline, isoleucine, leucine, methionine, phenylalanine, proline, serine, threonine, tryptophane, tyrosine, valine) (Singh and Brown 1957). In these experiments osmotic pressure was varied by adding different amounts of an amino acid mixture in the above mentioned diet. Thus the diets having osmotic activity between $\triangle$ 0.15°C to $\triangle$ 0.52°C. or equivalent to 0.23 to 0.78 per cent NaCl were obtained.

Results

The effect of different osmotic pressures of the diet medium is represented in Table I and Table II, in which average time in each instar with standard error is given. In both experiments it is very clear that growth and development of mosquito larvae can take place if the osmotic pressure is below $\triangle$ 0.40°C. Above this limit there is no growth. In the second set of experiments optimum growth was obtained at $\triangle$ 0.39°C. There was no growth above this level and only poor growth below it. The reason for the failure of growth at lower osmotic pressures was not due to the effect of osmotic pressure *per se*, but rather was due to the insufficient dietary amino acids.

Discussion

From these experiments it is quite evident that the osmotic pressure of the diet has great effect upon the growth and development of *A. aegypti*. If this larva has to be grown on a diet, which has all the ingredients in solution, then the number of molecules in the solution must be restricted, so that the osmotic pressure of the diet does not exceed $\triangle$0.40°C.

Wigglesworth (1933) reported that larvae of *A. aegypti* grew normally if the concentration of NaCl was below 0.8 per cent, and the artificial sea water up to a concentration equivalent to 1.1 per cent NaCl. The death of the larvae at higher concentration was not due to the higher osmotic pressure but was due to the presence of directly toxic concentrations of certain inorganic ions. As he was studying the effect of only salts in the solution and giving the remainder of the nutrient as insoluble factor, he could not separate the effect of salts and osmotic pressure. In the present series of experiments special care was taken to maintain salts and other ingredients in the diet below the toxic level. Also the osmotic

TABLE I

The Effect of Osmotic Pressure of the Diets on the Growth and Development of *A. aegypti* Larvae. Number of larvae completing each instar (n) and days spent in each instar (t) Basic diet:—Insoluble residue of yeast, salt mixture, RNA, vitamins and glucose*

Glucose gm/1	Freezing point depression △, °C.	No. Inoc.	Instar I		Instar II		Instar III		Instar IV		Pupal Instar		Total Aver. Time days	Range Days
			n	t	n	t	n	t	n	t	n	t		
5.0	0.12	20	16	2.2 ±0.25	16	1.2±0.19	16	1.0±0.13	14	2.6±0.16	13	2.0±0.00	9.1 ±0.31	8.0-11.5
10.0	0.17	20	20	2.0 ±0.02	20	1.2±0.02	19	1.3±0.01	19	2.7±0.03	18	2.1±0.02	9.34±0.33	8.5-13.0
20.0	0.29	20	20	2.27±0.19	20	1.5±0.13	20	1.5±0.07	16	3.5±0.12	16	2.0±0.03	11.31±0.25	10.0-14.0
30.0	0.37	20	15	1.9 ±0.53	15	2.0±0.39	15	1.5±0.17	10	4.3±0.41	8	2.4±0.16	11.25±0.16	10.5-12.0
35.0	0.45	20	—	No growth										
40.0	0.55	20	3	3.6 ±0.64	1	2.0±0.00	0	—	—	—	—	—	—	—
50.0	0.68	20	0	No growth										
60.0	0.74	20	0	No growth										
80.0	0.98	20	0	No growth										

* Osmotic pressure of the diet was changed by adding different amount of glucose to the diet.

TABLE II

The Effect of Osmotic Pressure of the Diets on the Growth and Development of *A. aegypti* Larvae
Number of larvae completing each instar (n) and days spent in each instar (t)
Basic diet:—Glucose, salt mixture, RNA, vitamins, glutathione, lipids and amino acids*

Amino Acids gm./1	Freezing point depression △, °C.	No. Inoc.	Instar I		Instar II		Instar III		Instar IV		Pupal Instar		Total Aver. Time Days		Range Days	
			n	t	n	t	n	t	n	t	n	t				
2.0	0.15	20	0	No growth												
4.0	0.23	20	6	13.0±0.14	0	—	—	—	—	—	—	—	—	—	—	—
8.0	0.29	20	19	8.5±0.80	14	6.4±0.48	3	6.0±0.13	0	—	—	—	—	—	—	—
11.64	0.39	20	20	2.9±0.04	20	2.6±0.05	20	3.0±0.22	16	5.6±0.35	16	2.0±0.00	15.0±0.42		13.0-19.0	
16.0	0.44	20	12	7.0±0.52	3	10.0±0.47	0	—	—	—	—	—	—	—	—	—
22.0	0.52	20	0	No growth												

* Osmotic pressure of the diet was changed by adding different amount of amino acid mixture.

pressure of the diet medium was varied by two different components of the diet to ensure that there was no direct interference on the larval growth and development. It is very clear therefore, that osmotic pressure does have an effect on the larval growth and development of *A. aegypti*.

Moreover, the haemolymph osmotic pressure of *A. aegypti* is equivalent to 0.75 to 0.89 per cent NaCl. The larvae can maintain a constant osmotic pressure of the haemolymph in ambient solutions equivalent to 0.75 per cent NaCl or less although they do not have the ability to do so in hypertonic solutions (Wigglesworth, 1938). *Culex pipiens* also shows the same kind of increase in the haemolymph osmotic pressure in hypertonic solutions. On the other hand, *Aedes detritus* being a salt marsh mosquito, has the ability to maintain a moderate haemolymph osmotic pressure even when the ambient concentration reaches 6.0 per cent NaCl, and a haemolymph concentration not exceeding 1.3 per cent NaCl, (Beadle, 1939). *Culex fatigans* has similar abilities (Woodhill, 1938). Since mosquito species like *A. aegypti* cannot maintain their haemolymph osmotic pressure and are unable to absorb food through their midgut in hypertonic solutions, they do not grow but die at higher osmotic pressures.

Summary

The osmotic pressure of the diet has a great effect on the growth and development of *A. aegypti*, when all the nutrients are in the solution form. The growth and development of these mosquito larvae can continue only if the osmotic pressure of the diet is at or below $\triangle$ 0.40°C., provided the nutrient materials are in sufficient amount. Above this osmotic pressure the larvae do not grow.

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RESULTS OF GASTRIC EXAMINATION BY FRACTIONAL METHOD

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IN various disorders of the stomach, examinations on its secretory functions are usually investigated by a " fractional gastric analysis ". The fasting juice is completely evacuated and a test meal is introduced into the stomach, which will stimulate the gastric cells. The response to this stimulation is then measured clinically by the determination of total acidity, free acidity, presence or otherwise of blood, bile, starch, mucus, lactic acid etc. in the stomach contents, which are periodically withdrawn. Many forms of test meal have been used. The most commonly used test meals consist of either an oatmeal gruel or 50 cc. of 7% ethyl alcohol.

On obtaining an achlorhydric response, further investigations become necessary to distinguish the condition of pseudo-achlorhydria from true achlorhydria. In this latter condition, known as true achylia gastrica, which is associated with pernicious anaemia, neither free hydrochloric acid nor enzymes are secreted, even after most powerful provocation. Histamine is the most powerful stimulant of gastric secretion known and helps to unmask, the acid, even if there is a trace of the secretory activity of the stomach left. It produces, a juice of maximal acidity and relatively low pepsin content. With histamine stimulation, the free acidity rises rather promptly, reaching a maximum usually in forty to sixty minutes and then declining. The volume of gastric juice varies correspondingly.

In achlorhydreas following gastritis or malignancy, a low acid secretion is revealed on injecting histamine. Histamine acts by stimulating the oxyntic cells of the gastric glands. Thus " Histamine meal " examination becomes an important test of the secretory power of the stomach under standard but unphysiological conditions.

Some investigations were carried out here in the Bio-chemical Laboratory, on some of the patients with gastric disorders, by examination of their gastric secretory function, with alcohol and/or histamine meal tests. In order to have a comparable data, some normal subjects were studied by the fractional method. The results of these investigations indicating

the importance of this test over that of the alcohol meal test observed in some of the patients studied for their gastric disorders have been presented in this paper.

Material and Methods

Twenty persons of both sexes were tested by the standard fractional gastric analysis method. Out of these, ten were normal subjects who were male medical students of age between 18 and 22 years and the rest were patients, admitted in the wards of S. S. G. Hospital, Baroda. Of the latter group, half were males and the remaining half females. They ranged in their age from 15 to 52 years. The fractional gastric analysis was done after the alcohol meal according to the method of Bloomfield and Keefer (1927). For the histamine test meal examination, the method of Bloomfield and Polland (1929) was adopted with modifications. The resting juice was completely aspirated and the stomach washed with several syringfuls of warm water until clean rinsing fluid was obtained. Histamine was then injected subcutaneously in dosage of 0.01 mg. per kilo of the body weight, after ascertaining that the systolic blood pressure of the subjects was above 110 mm. of Hg. Subsequently the gastric samples were aspirated periodically. These samples were then analysed for their free and total acidity content and were examined for the presence or otherwise of blood, bile, starch, mucus and lactic acid.

Results

In the normal subjects, the highest free acidity obtained in any of the samples aspirated after the alcohol meal ranged between 20 to 106 cc. of N/10 acid%. In case of the patients the highest free acidity observed in any of the samples aspirated after the histamine meal was found to range between 0 and 58cc. of N/10 acid% (vide table).

Discussion

Napier, Chaudhari and Rai Choudhari (1938) established the following criteria for classifying acid curves according to highest free acid readings per 100 c.c. of gastric juice :—

(1)	Achlorhydria	0
(2)	Hypochlorhydria	less than 10
(3)	Isochlorhydria (low)	10 to less than 25
	—do—(medium)	25 to 45
	—do—(high)	45 to 65
(4)	Hyperchlorhydria	greater than 65.

TABLE

Highest free acidity after alcohol and histamine meals

Sr. No.	Subject	Age in years	Sex	Free acidity in cc. N/10 acid % Range	highest	Remarks
1.	S. K. C.	10	M.	26-84(A)	84(A)	Normal Subject
2.	P. C. F.	21	M.	34-74(A)	74(A)	do
3.	K. V. R.	22	M.	26-58(A)	58(A)	do
4.	V. A. C.	20	M.	0-20(A)	20(A)	do
5.	M. K. B.	19	M.	40-106(A)	106(A)	do
6.	D. V. M.	19	M.	14-40(A)	40(A)	do
7.	P. R. D.	22	M.	16-82(A)	82(A)	do
8.	V. B. S.	18	M.	0-36(A)	36(A)	do
9.	P. S. B.	18	M.	16-56(A)	56(A)	do
10.	B. M. R.	20	M.	40-65(A)	65(A)	do
11.	P. U. A.	25	M.	(0 (A) (24-58(H)	(0 (A) (58 (H)	Patient
12.	J. H.	40	F.	(0 (A) (0 (H)	(0 (A) (0 (H)	,,
13.	U. I.	44	M.	0 (A)	0 (A)	,,
14.	D. M.	20	F.	(0 (A) (0-30 (H)	(0 (A) (30 (H)	,,
15.	K. N.	16	F.	0 (A)	0 (A)	,, (Ova of Ankylostoma found in stools).
16.	A. S. D.	23	M.	(0 (A) (0 (H)	(0 (A) (0 (H)	Patient
17.	B. F.	20	M.	(0 (A) (0 (H)	(0 (A) (0 (H)	(Ova of Ankylostoma found in stools).
18.	D. B.	52	M.	(0 (A) (0 (H)	(0 (A) (0 (H)	do
19.	A. D.	15	F.	(0 (A) (0 (H)	(0 (A) (0 (H)	do
20.	G. A.	16	F.	(0 (A) (0-60 (H)	(0 (A) (60 (H)	do

A: After "Alcohol meal".

H: After "Histamine meal".

According to this classification, out of the ten normal subjects, five were with hyperchlorhydric response, four with high isochlorhydric response and the remaining one was with low isochlorhydria.

The quantity of the fasting juice aspirated in these subjects ranged between 4 c.c. to 94 cc. with an average of 39.8c.c. The volume of the fasting juice has been reported to be varying within wide limits. In the

previous communications made from this laboratory, the average volume of the fasting juice observed was 37.17 cc. (Pathak and Pai, 1954 and 1956). In none of the fasting samples was there evidence of lactic acid or of foul odour, which are indicative of fermentation in the stomach. Taking all samples together mucus was present in about 60% and bile in about 40% but no sample showed evidence of blood or lactic acid.

The patients who were studied for their gastric analysis examinations were admitted mostly for the complaints of either pain and/or burning sensation in the epigastrium, accompanied by anaemia. The fractional gastric analysis after the alcohol meal showed in all these cases an achlorhydric response. Out of the ten cases, in seven the fractional gastric analysis examination was possible to be repeated on subsequent occasions before any treatment given, with histamine meal instead of the alcohol meal, in order to determine the nature of achlorhydria, that is, whether true or false. In three cases out of these seven, in whom the histamine meal was given, the highest free acid was found to be ranging between 30 to 60 c.c. N/10 acid %.

In the remaining four cases, however, the achlorhydria obtained after the alcohol meal, was confirmed by the histamine meal test. By means of the latter test, thus it was possible to distinguish the true achlorhydria from the false one.

Another interesting observation made in this study of the patients, is that the occurrence of achlorhydria as determined above has coincided, in five of the ten cases, with the presence of ova of Ankylostoma in their stools, on being examined.

In the patient K. N. (S. No. 15), with ankylostomiasis, the achlorhydria as obtained by the alcohol meal test could not be confirmed subsequently by the histamine meal test, whereas in the patient G. A. (S. No. 20), the achlorhydria as determined by the alcohol meal examination was subsequently found to be pseudo-achlorhydria when examined by the histamine test.

It is mostly for the symptoms of anaemia that the majority of persons harbouring Ankylostoma seek medical treatment. Even though they may be apparently healthy and carrying out their routine work, they suffer from anaemia and their blood level is far below the normal count. Hook-worm, however, can exist in human beings without producing anaemia, and hookworm can live in the intestines without producing any

other signs and symptoms in a stage of symbiosis. Probable causes of absence of anaemia in these cases might be a light infection, adequate nutrition, immunity between the host and the parasite etc. (Patel, 1954). There are various explanations given by various workers for the hookworm anaemia, which may be :—(1) loss of blood, (2) toxins which may damage the red blood cells or the marrow which produces them or (3) faulty metabolism. Thus Napier *et al* (1941) considered hook-worm anaemia mainly due to blood loss but they indicate that there is some other minor factor, such as failure of absorption as a result of intestinal mucosal dysfunction.

One of the probable causes for the presence of achlorhydria in some of the patients, studied in the present series, who were diagnosed as suffering from ankylostomiasis, may be similarly sought in the faulty metabolism connected with the inadequate absorption, which may possibly lead to the condition of deficiency of certain factors in the person. Evidence is available, from experiments performed on animals to show the effect of certain B Vitamin deficiencies on gastric secretion, that the gastric secretion is sharply reduced by pyridoxine deficiency (Hawk and Hundley, 1951).

Of course, it must be admitted that before any definite causative relationship could be established between the occurrence of achlorhydria and ankylostomiasis, further work is necessary.

Summary

(1) Twenty persons of both sexes varying in their age from 15 to 52 years have been examined with the fractional gastric analysis method employing alcohol and histamine meals.

(2) Out of seven patients who gave achlorhydric response with alcohol meal three showed, on subsequent examination with histamine meal, an isochlorhydria (medium or high) response.

(3) Thus " histamine meal " examination becomes an important test for the secretory power of the stomach under standard but unphysiological conditions.

(4) In five out of ten patients, it was observed that the occurrence of achlorhydria coincided with the presence of ova of Ankylostoma in their stools.

(5) The significance of the above findings is discussed.

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THE EFFECT OF VARIOUS SUBSTANCES ON THE INTESTINAL PROTEINASE OF THE EARTHWORM, *PHERETIMA ELONGATA* (PERRIER)

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IN a previous paper (Kamat, 1955), a report on certain characteristics of the intestinal proteinase of the earthworm, *Pheretima elongata* (Perrier) has been given. Here a study of the effect of certain substances on the activity of this enzyme has been made, with a view to compare this enzyme with the corresponding ones from other sources.

Materials and Methods

Casein was used as substrate throughout this investigation. The digests were buffered at pH 8.7 using Michaelis buffer (1931); the solutions of various activators/inhibitors were also, wherever necessary and practicable, adjusted to pH 8.7. The only exceptions were those salts which formed precipitates at this pH. One of the following two enzyme preparations were used :—(a) a Veronal acetate buffer extract of powdered enzyme; and (b) a freshly prepared aqueous enzyme extract. In each case, the reaction was followed by estimating the entire contents of the digestion vessel to obtain a single reading using the method of White and Bowman (1947). The preparation of the substrate and enzyme, and the method of estimation have been previously described in detail (Kamat, 1955).

The figures obtained and given in the tables represent relative velocity i. e. the initial velocity as percentage of the initial velocity in the control digest.

Results

Effect of Amino-acids: The effect of some of the common amino-acids and asparagine, on the proteinase activity was studied at pH 8.7. The enzyme preparation was an aqueous extract of the enzyme powder. The results are given in Table No. I.

In these experiments it was found that leucine, histidine and phenylalanine have indifferent action on the activity of the proteinase under

investigation. Cysteine hydrochloride slightly activates the enzyme, whereas glutamic and aspartic acids and asparagine, show substantial increase in the activity of the enzyme. This fact has an additional significance in that the native proteins accompanying the proteinase may contain amino-acids which have a profound effect on the course and pace of the activity of the enzyme.

Effect of Salts. Here the effect of the addition of various salts on the activity of the proteinase was examined. In carrying out these experiments the solution of the salt was first added to the freshly prepared aqueous extract of the enzyme. This enzyme salt mixture was allowed to stand for 20 minutes at 37°C in an incubator. It was then added to the substrate-buffer solution. At higher concentrations calcium chloride and lead acetate developed slight precipitates on standing. Excepting that sodium nitroprusside gave a light purple colour to the enzyme solution, there was no other significant change. The results are given in Tables II and III. The concentrations of the salts given in the tables, are those in the salt-enzyme solutions before addition to the buffered substrates. The concentrations in the digest mixtures were one-sixth of these recorded values.

As can be seen from Table II, the salts of heavy metals neither activate nor inhibit the activity of this proteinase. On the other hand, it is seen from Table III that as with papain, in this case also, the cyanides, sodium cyanide and potassium ferro—and ferri-cyanides have a distinctly accelerating effect. This confirms similar results obtained in the case of the pancreatic proteinase (Farber and Wynne, 1935). Similarly calcium chloride, in the concentrations used, is also found to have an activating effect on the intestinal proteinase of earthworm. This also corroborates the work of Farber and Wynne.

On the other hand, sodium fluoride, sodium nitroprusside and magnesium sulphate do not have any significant effect on the activity of this proteinase.

Effect of Glycerol. The enzyme solution used in this case was an aqueous extract of the enzyme powder. Results are given in Table IV.

The results show that in the moderately high concentrations used, glycerol has an inhibitory effect on the enzyme. The inhibition is greater with high concentrations and is comparatively less with low concentrations. This corroborates the fact that aqueous glycerol extract of the enzyme, wherever used, is found to lose much of its activity after some time.

Effect of Sugars. Six of the well known sugars were used in these experiments. Stock buffered solutions of known molar concentration were added to the digestive mixtures to give overall concentrations listed in Table V. The enzyme solution was a Veronal-acetate buffer extract of the powdered enzyme. Results are entered in Table V.

The results show that sugars in the concentrations used have a marked inhibitory action on the enzymic activity and is greatest at higher concentrations, whereas in many cases, nearly negligible at low concentrations.

Effect of Polysaccharides. Stock buffered solutions of dextrin, starch and gum acacia of 18% concentration were used to determine their effect on the activity of the enzyme. These solutions were added to the digestive mixtures in such amounts that the concentrations given in Table VI could be obtained. In carrying out these experiments Veronal-acetate buffer extracts of the enzyme powder were used. Results are shown in Table No. VI.

The results show that in the case of dextrin and starch, inhibition was less marked compared to the sugars where it was greatest at high concentrations and negligible at low concentrations.

Gum acacia has no significant effect on the activity of the enzyme as it neither accelerates nor inhibits.

Effect of Bile and Bile Salts. Ringer (1921 and 1922) Vonk, *et al* (1933), Farber and Wynne (1935) and others observed that in an alkaline solution bile and bile salts diminish the rate of breakdown of proteins by trypsin. Willstatter and Persiel (1925) on the other hand maintain that sodium glycocholate in 0 002 M concentration has no effect on tryptic activity determined by the increase in acid titrable in alcoholic solution. The effect of fresh ox bile and the two bile salts, sodium taurocholate and sodium glycocholate on the protein liquefying activity of the intestinal proteinase of the earthworm were determined, the results of which are given in Tables, VII and VIII.

From these results it could be inferred that fresh ox bile and sodium taurocholate and sodium glycocholate (the last named even at as low a concentration as 0.001 M) have a distinctly inhibitory effect on the initial activity of this enzyme.

Effect of Indicators and Dye-Stuffs. The effect of some indicators and dye-stuffs on the initial activity of the intestinal proteinase of the earth-

worm was determined by the usual methods. Here the solution of the substance was first mixed with the freshly prepared aqueous extract of the enzyme, allowed to stand for 20 minutes at 37°C in an incubator, and then this mixture was added to the substrate-buffer solution, the initial activity of the enzyme being determined as usual.

The substances chosen for the investigation were the following :— bromothymol blue, methyl orange and phenol-phthalein representing the indicator group ; congo red (acidic reaction) and bismarck brown (basic reaction) representing tetrazo-dyestuffs ; whereas, safranines and thiasines were represented by safranine (basic reaction) and methylene blue (basic reaction) respectively. These substances form comparatively a representative group and include dyestuffs having acidic and basic reactions.

The results are summarised in Table IX. The concentrations of the indicators/dyestuffs mentioned in the table are those in the indicator/dyestuff-enzyme mixtures before addition to the buffer-substrate solutions. Hence the concentrations in the digest mixtures were one-sixth of their recorded values.

The results show that in the concentrations used the indicators and dyestuffs were quite inert towards the intestinal proteinase of the earthworm. These results appear to support the work of Marston (1923) who showed that the proteolytic enzymes are specifically precipitated by dyes of the safranine type, the compounds produced still retaining proteolytic activity.

Discussion

In the present investigation the effect of various substances on the activity of the intestinal proteinase of the earthworm *Pheretima elongata* (Perrier) has been studied. Although in these experiments the effect of all the known activators/inhibitors of the various proteinases have not been compared, yet these experiments are sufficient to indicate the existence of a very close similarity between the intestinal proteinase of the earthworm and the proteinases from other animal sources.

The investigation reveals that in general the activators of the mammalian pancreatic trypsin activate this enzyme while the inhibitors of the former likewise inhibit it. Also in view of the fact that this proteinase hydrolyses casein optimally in the alkaline region (Kamat, 1955), it presents a great similarity between the two hydrolytic enzymes from such widely different groups of animals. Yet a closer study reveals certain

characteristic differences also. Thus as shown previously (Kamat, 1955), this enzyme does not respond to enterokinase, the specific activator of trypsin. This may be either due to the fact that the earthworm proteinase is not of tryptic nature or else that unlike trypsin it is already in an active state. On the whole it appears to be more satisfactory to avoid classifying this intestinal proteinase of earthworm with any of the three main groups of proteinases but rather leave it in a class by itself.

Summary

The effect of various substances on the enzyme activity was studied and the following observations were made :—

(1) The amino-acids cysteine-HCl, aspartic acid and glutamic acid, and the peptid, asparagine were found to accelerate the action of the enzyme, whereas other amino-acids showed no effect.

(2) Salts of heavy metals were found to have no effect on the enzymic activity.

(3) Sodium cyanide, potassium ferro- and ferricyanide, and calcium chloride showed a profound accelerating effect ; other salts like sodium fluoride, sodium nitroprusside and magnesium sulphate were indifferent.

(4) Glycerol acted as an inhibitor of this enzyme.

(5) Sugars too showed a marked inhibitory effect.

(6) Of the polysaccharides tried, dextrin and starch were less inhibitory, while gum acacia was indifferent.

(7) Bile and bile salts were found to be inhibitors.

(8) Indicators and dyestuffs were indifferent.

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TABLE NO. I

Effect of Certain Amino-acids and Asparagine on the Activity of the Intestinal Proteinase of the Earthworm

(The figures represent relative velocity *i.e.* the initial velocity as percentage of the initial velocity in the control digest)

	Substance	Concentration M						
		0.0015	0.0031	0.0062	0.0125	0.025	0.50	1.00
1	Leucine	—	98	—	—	97	—	99
2	Histidine	99	—	100.4	—	97	—	—
3	Phenylalanine	—	100	—	100.4	99	—	96.7
4	Cysteine-HCl	99	—	100	—	106	—	103
5	Glutamic acid	101	—	102	—	107	125	—
6	Aspartic acid	101	—	104	—	112	126	—
7	Asparagine	—	102	—	109	—	—	132

TABLE NO. II

Effect of Salts of Heavy Metals on the Activity of the Intestinal Proteinase of the Earthworm

(The figures represent relative velocity *i.e.* the initial velocity as percentage of the initial velocity in the control digest)

	Substance	Concentration M $\times 10^{-4}$						
		0.55	1.09	2.19	4.38	8.75	17.5	35.0
1	Silver Nitrate	96	—	99	—	101	—	100
2	Mercuric Chloride	105	—	101	—	100	—	102
3	Lead Acetate	102	—	105	—	105	—	102
4	Cupric Chloride	—	102	—	101	—	—	103

TABLE No. III

Effect of various Salts on the Activity of the Intestinal Proteinase of the Earthworm

(The figures represent relative velocity *i.e.* the initial velocity as percentage of the initial velocity in the control digest)

	Substance	Concentration M × 10^{-4}						
		0.55	1.09	2.19	4.38	8.75	17.5	35.0
1	Sodium Fluride	—	101.8	—	100.9	—	98	—
2	Sodium nitroprusside	100	—	101.8	—	97.7	—	98
3	Magnesium Sulphate	—	98	—	—	99	97	100
4	Sodium cyanide	112.8	—	—	129	133	—	105
5	Potassium ferrocyanide	107	—	126	—	106	—	105
6	Potassium ferricyanide	107	—	125	—	112	—	109
7	Calcium Chloride	118	—	128	—	109	—	105

TABLE No. IV

Effect of Glycerol on the Activity of the Intestinal Proteinase of the Earthworm

	Concentration M.	Relative Velocity
1	Nil	100
2	0.22	93
3	0.45	84
4	0.90	65
5	1.35	45
6	1.8	39

TABLE No. V

Effect of Sugars on the Activity of the Intestinal Proteinase of the Earthworm

(The figures represent relative velocity *i.e.* initial velocity as percentage of the initial velocity in the control digest)

	Sugars	Concentration M					
		0.015	0.031	0.062	0.125	0.25	0.50
1	Fructose	—	73	76	—	62	56
2	Galactose	93	—	81	—	70	64
3	Glucose	93	88	—	—	73	55
4	Lactose	97	92	—	—	79	64
5	Maltose	—	95	—	85	80	60
6	Sucrose	100	—	—	91	79	62

TABLE No. VI

Effect of Polysaccharides on the Activity of the Intestinal Proteinase of the Earthworm

(The figures represent relative velocity *i.e.* the initial velocity as percentage of the initial velocity in the control digest)

	Substance	Concentration M					
		0.28	0.56	1.12	2.25	4.5	9.0
1	Dextrin	99	—	94	—	87	—
2	Starch	101	98	—	82	—	76
3	Gum acacia	—	97	—	99	—	102

TABLE No. VII

Effect of fresh ox bile on the Activity of the Intestinal Proteinase of the Earthworm

	% Concentration of bile	Relative velocity
1	Nil	100
2	0.10	94
3	1.0	90
4	10.0	63
5	20.0	20.5
6	30.0	17

TABLE No. VIII

Effect of Bile Salts on the Activity of the Intestinal Proteinase of the Earthworm

	Substance	Concentration M			
		0.001	0.01	0.02	0.03
1	Sodium Glycocholate	83.5	73	62	47
2	Sodium Taurocholate	62	46	—	32

TABLE No. IX

Effect of Indicators and Dyestuffs on the Activity of the Intestinal Proteinase of the Earthworm

(The figures represent the relative velocity *i.e.* the initial velocity as percentage of the initial velocity in control digest)

	Substance	Concentration M $\times 10^{-4}$						
		0.22	0.45	0.90	1.8	3.6	18	36
1	Bromothymol blue	—	—	105	107	—	109	—
2	Methyl orange	—	—	96	—	98.6	101	—
3	Phenolphthalein	—	—	96.8	94	—	92.6	—
4	Bismark brown	—	—	—	96	99	—	102
5	Congo red	99	94	—	95	—	96	—
6	Methylene blue	99	94	—	95	—	—	—
7	Safranine	—	—	99	106	—	104	—

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